ENCE LIBRARY
MAY 1 1933

VOLUME 23 NUMBER 4

APRIL, 1933

Phytopathology

An International Journal

Official Organ of
The American Phytopathological Society

CONTENTS

The nature of potato rugose mosaic ..... KARL LEE KOCH 319
Physiologic forms of wheat stem rust in Kansas and Nebraska. GEORGE L. PELTIER 343
The technique of isolation in microbiology ..... SYDNEY DICKINSON 357
Gibberella moniliformis on corn ..... R. K. VOORHEES 368
The production of a white fertile saltant of Helminthosporium sativum by means of ultra-violet radiation. F. J. GREANEY and J. E. MACHACEK 379
Occurrence and pathogenicity of Nematospora spp. in Florida. GEORGE F. WEBER 384
Application of the terms "anthracnose" and "scab" to plant diseases caused by Sphaceloma and Gloeosporium ..... ANNA E. JENKINS 389
Experiments with head smut of corn in western Oregon. E. N. BRESSMAN and H. P. BARSS 396
How plant pathology can contribute to a land-utilization program. NEIL E. STEVENS 404
Phytopathological Notes 407
A hitherto unreported blight of the Juglandaceae. ROBERT T. MORRIS
A disorder of Persian (English) walnuts grafted on black-walnut stocks, resulting in girdling. C. E. SCHUSTER and P. W. MILLER

Price { $6.00 per year, United State and dependencies,
$6.25 per year, Canada
$6.50 per year, elsewhere

PUBLISHED MONTHLY BY THE SOCIETY

OFFICE OF PUBLICATION

COR. LIME AND GREEN STREETS, LANCASTER, PA.

Entered at the post-office in Lancaster, Pa., as second-class matter.

DIRECTORY OF ADVERTISERS

LABORATORY EQUIPMENT AND SUPPLIES

BAUSCH & LOMB OPTICAL CO., 665 St. Paul St., Rochester, N. Y.	MICROSCOPES. MICROTOMES.
SPENCER LENS CO., Buffalo, N. Y.	MICROSCOPES.
CLAY-ADAMS CO., 25 East 26th St., New York, N. Y.	SLIDES AND COVER GLASSES. MICROSCOPIC PROJECTION AND DRAWING APPARATUS.
DIFCO LABORATORIES, 920 Henry St., Detroit, Mich.	DEHYDRATED CULTURE MEDIA.
WARREN-KNIGHT CO., 136 N. 12th St., Philadelphia, Pa.	WRICO LETTERING GUIDES AND DRAFTING SUPPLIES.

FUNGICIDES

AMERICAN CYANAMID CO., 535 Fifth Ave., New York, N. Y.	AERO CYANAMID FOR SOIL STERILIZATION AND WEED CONTROL.
ANSUL CHEMICAL CO., Marinette, Wis.	ANSUL FORMALDEHYDE DUST AND ANSUL COLLOIDAL SULPHUR.
THE GRASSELLI CHEMICAL CO., Cleveland, Ohio.	SPRAY MATERIALS AND DUSTS.
KOPPERS PRODUCTS CO., Koppers Building, Pittsburgh, Pa.	FLOTATION SULPHUR. PASTE, DRY WETTABLE, DUST.
ROHM & HAAS CO. INC., 222 W. Washington Square, Philadelphia, Pa.	RED COPPER OXIDE. FORM-O-FUME FORMALDEHYDE DUST.

SCIENTIFIC BOOKS AND PUBLISHERS

BIOLOGICAL ABSTRACTS, University of Pennsylvania, Philadelphia, Pa., U. S. A.	BIOLOGICAL ABSTRACTS
PHYTOPATHOLOGICAL CLASSICS H. H. Whetzel Mgr., Cornell University, Ithaca, N. Y.	NUMBERS 1 AND 2. PHYTOPATHOLOGICAL CLASSICS.
P. BLAKISTON'S SON & CO., 1012 Walnut St., Philadelphia, Pa.	FOUR NEW BOOKS: RECENT ADVANCES IN BOTANY, PLANT GENETICS, PLANT PHYSIOLOGY, CYTOLOGY.
SCIENCE PRESS, Lime and Green Sts., Lancaster, Pa.	PRINTERS OF SCIENTIFIC JOURNALS, BOOKS AND MONOGRAPHS.
JOHN WILEY & SONS, INC., 440 Fourth Ave., New York, N. Y.	J. C. ARTHUR PLANT RUSTS
THE H. W. WILSON CO., 950-972 University Ave., New York, N. Y.	CLEMENTS AND SHEAR. THE GENERA OF FUNGI.

HOTELS

MADISON HOTEL Atlantic City, N. J.	HOTEL.

FERTILIZERS

N. V. POTASH EXPORT M. Y., INC., 19 West 44th St., New York, N. Y.	AGRICULTURAL AND SCIENTIFIC BUREAU. POTASH FERTILIZER.

PHYTOPATHOLOGY

VOLUME 23 NUMBER 4

APRIL, 1933

THE NATURE OF POTATO RUGOSE MOSAIC

KARL LEE KOCH[1]

(Accepted for publication July 13, 1932)

INTRODUCTION

Within the past 15 years the potato has been shown to be subject to many different virus diseases. That some of these, however, might be due to a complex of 2 or more distinct viruses has been recognized for some time. Recent investigations showing that some potato viruses are transmissible to related host plants have stimulated closer attention to the viruses themselves as distinct entities. Additional methods for separating plant viruses have enabled a more careful scrutiny of the potato-virus diseases for possible virus combinations, which may occur in nature as typical diseases. Potato rugose mosaic, especially, was a source of considerable speculation relative to its nature and behavior. This disease is so constituted that it is almost as obscure as any infectious disease with which plants are affected. The nature of the disease has, therefore, come to light only very gradually through the contributions of several different workers. The present investigation of rugose mosaic was started, and its dual nature established, before it was known that other workers had arrived at and published similar conclusions. Several phases were in need of clarification, however, and with this object in view the problem was expanded and continued, the results of which are here presented.

REVIEW OF LITERATURE

Atanasoff's (3) review of early descriptions of potato diseases of the type that are now believed to have been of virus origin indicates that this group of maladies has been recognized for some time. As far as can be clearly established from the literature, however, the conception of the virus nature of these potato diseases was first developed in 1914 by Orton (13) and Appel (2). It remained, however, for Schultz, Folsom, Hildebrandt,

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

The writer wishes to acknowledge his indebtedness to Dr. James Johnson for the opportunity to conduct these investigations under his direction and in his laboratory. He wishes to express his appreciation to Prof. H. F. Wilson, who has made possible the carrying on of this work.

and Hawkins (20) in 1919 to prove conclusively the relation of viruses to potato degeneration diseases. It soon became evident to Schultz and his coworkers (19) and almost simultaneously to Murphy (12) in Ireland and Quanjer (15) in Holland that several different viruses were concerned in these obscure potato maladies.

What may be termed the second period in the potato-virus problem became a study of the symptomatology of the diseases themselves, to which many investigators contributed. The particular disease with which we are concerned in this paper was named rugose mosaic by Schultz and Folsom (19) and was described as follows: "More infectious than 'mild mosaic' and differentiated by distinct dwarfing, more chlorosis and diffuse mottling, a more rugose type of wrinkling, and a tendency to show brittleness, spotting, streaking, leaf-dropping and premature death. Tuber symptoms are a marked reduction in size." What is probably the same disease was called crinkle by Murphy (11) and Quanjer (14).

The third period of development, regarding rugose mosaic, may be said to have started in 1925 when Johnson (7) demonstrated the presence of a virus in all apparently healthy potatoes of most standard American varieties. This virus known as the mottle virus was, however, sometimes associated in apparently healthy potatoes with what appeared on the basis of symptoms to be another virus, named by Johnson ring-spot (7). Johnson's results indicated that the mottle virus could be increased in virulence by passage through tobacco, producing a "spot-necrosis" disease of tobacco and a severe disease of potatoes. This spot-necrosis of tobacco was later recognized by him (9) to be identical with potato rugose mosaic.

It was at this point that the writer (10) took up the studies in Johnson's laboratory and showed definitely that there were actually 2 distinct viruses concerned in rugose mosaic of potato or spot-necrosis of tobacco, the one being the mottle virus present in apparently healthy potatoes and the other an insect-transmitted virus.

However, it soon became evident that, prior to the writer's studies, K. M. Smith (21), in England, had worked along the same lines with a disease referred to as potato mosaic, although he did not recognize the dual nature of this disease until 1931 (22).

Valleau and Johnson (23), of Kentucky, working at about the same time as Smith, claimed to have separated the 2 viruses producing rugose mosaic through the medium of nitrogen-starved tobacco plants and that the 2 viruses were found independently in nature. They recognized one of these to be the same as Smith's insect-transmitted virus, although they apparently did not carry on experiments in aphid transmission. Smith did not apply a satisfactory name to his insect-transmitted virus, and, as Valleau and Johnson applied the name veinbanding virus to this entity,

the use of this name may serve as well as any other, unless questions of priority in nomenclature arise.

The recent related work of others, such as that of Salaman (17, 18) and Quanjer (16), should also be taken into consideration. The puzzling potato-virus combinations and their differentiation are given extensive consideration, but, until more is known about the identity of the particular viruses used by different workers, it is not easy to correlate their work with that of others on the basis of published results only.

EXPERIMENTAL METHODS

The experiments on potato rugose mosaic were conducted under greenhouse conditions during the fall, winter, and spring months, as the environment and other conditions of the experiments can be more carefully controlled than in the field. Two greenhouses, one running at a lower temperature (17–22° C.) and one at a higher (27–32° C.), were available for the work.

The Bliss Triumph variety of potato was used in almost all experiments as the standard stock. The tubers were from indexed stocks, essentially free from the common virus diseases, though they all carried the mottle virus, normally present in apparently healthy American potatoes. The potatoes were grown in the cool house in rich compost soil and produced short, stocky, vigorous plants used for inoculation purposes.

The Connecticut Havana No. 38 strain of tobacco was used as the standard variety in all experiments. The plants were grown in the warm greenhouse, where the symptoms were produced more readily than in the cool house. Other solanaceous hosts were also grown in the high-temperature house. The tobacco was grown in new compost soil in 4-in. pots. To insure against any virus contamination from the pots themselves, they were previously steam-sterilized. The greenhouses were fumigated once a week to guard against aphid infestation.

The inoculum was obtained by crushing foliage of infected plants in a small sterile mortar, then straining the juice through cheesecloth. The potato plants were inoculated, when 2 to 5 in. high, by rubbing the upper surfaces of 3 young leaflets with a small piece of cheesecloth soaked in the prepared inoculum. A combination of the rubbing and pricking methods was used for inoculating the tobacco plants. This was done with a sterile needle, wrapped at the end with a wad of absorbent cotton to aid in carrying the inoculum, by pricking the 2 oldest leaves of the plant several times along the midrib and then rubbing or wiping the entire upper surface of the 2 leaves with the same needle. Other solanaceous hosts, excluding the potato, were inoculated and treated in the same manner as were the tobacco plants.

For insect-transmission experiments, the peach aphid, *Myzus persicae* Sulz., and the green potato aphid, *Macrosiphum solanifolii* Ashmead, were used as vectors. The green potato aphids were colonized upon young potato plants, while the peach aphids were colonized upon cabbage plants, suitable hosts for the aphids. As has been shown by Smith (22), as well as by our own trials, the aphids do not transmit the mottle or ring-spot viruses from potato, and there is no reason to believe that cabbage is susceptible to any of the viruses used. The method followed in these experiments was similar to that used by Hoggan (6).

The properties of the viruses were determined by using the methods employed by Allard (1) and Johnson (8). The inoculum used in these studies was obtained from infected tobacco plants.

EXPERIMENTAL RESULTS

Separation of the Viruses Causing Rugose Mosaic. The transmission of rugose mosaic (Fig. 1, B) by aphids was first reported by Schultz and

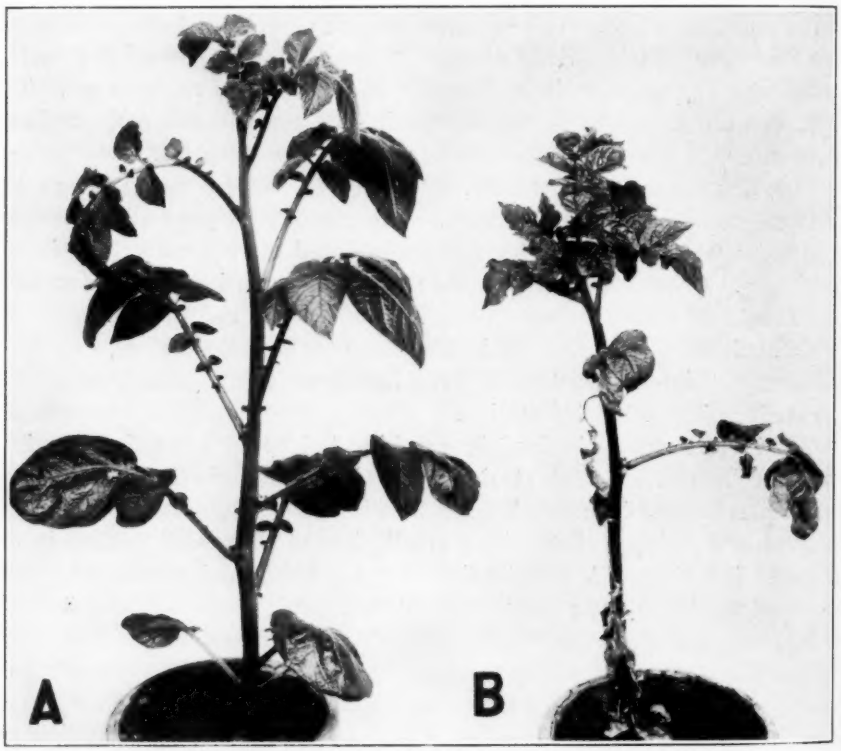

FIG. 1. Bliss Triumph variety of potato. A. Noninoculated control. B. Affected by rugose mosaic.

Folsom (19), who obtained apparent transmission of this disease by aphids from potato to potato. In the present investigation the writer also has readily obtained apparent transmission of this disease from potato to potato by means of the aphids *Myzus persicae* and *Macrosiphum solanifolii* (Table 1). Apparent transmission of the disease to potato was obtained

TABLE 1.—*Summarized results comparing aphid and artificial transmission of potato rugose mosaic to potato and tobacco*

Host inoculated	Source of inoculum	Inoculation by	No. of plants inoculated	No. developing symptoms of		Uninoculated controls	
				Rugose mosaic or spot-necrosis	Vein-banding only	No. of plants	No. devel. symptoms
Potato	Rugose mosaic on potato	Plant extract	15	13	0	5	0
		Myzus persicae	93	57	0	90	0
		Macrosiphum solanifolii	95	25	0	90	0
	Spot-necrosis on tobacco	Plant extract	40	33	0	25	0
		Myzus persicae	26	19	0	20	0
Tobacco	Rugose mosaic on potato	Plant extract	60	45	0	10	0
		Myzus persicae	29	0	27	25	0
		Macrosiphum solanifolii	38	0	23	35	0
	Spot-necrosis on tobacco	Plant extract	60	59	0	25	0
		Myzus persicae	35	0	35	30	0

also from tobacco by means of *Myzus persicae*. However, when an attempt was made to transmit rugose mosaic by means of aphids to tobacco, from either potato or tobacco, none of the aphid-infested tobacco plants developed symptoms of spot-necrosis (Table 1). These plants, however, were abnormal. They developed faint symptoms of another type, which appeared mainly as a clearing of the veins and a general flattening of the plants. Such symptoms were characteristic of all tobacco plants similarly infested

with aphids from rugose mosaic hosts. As these symptoms are later shown to be caused by a virus, similar to the veinbanding virus described by Valleau and Johnson (23), this disease will be referred to in this paper as veinbanding (Fig. 2, D).

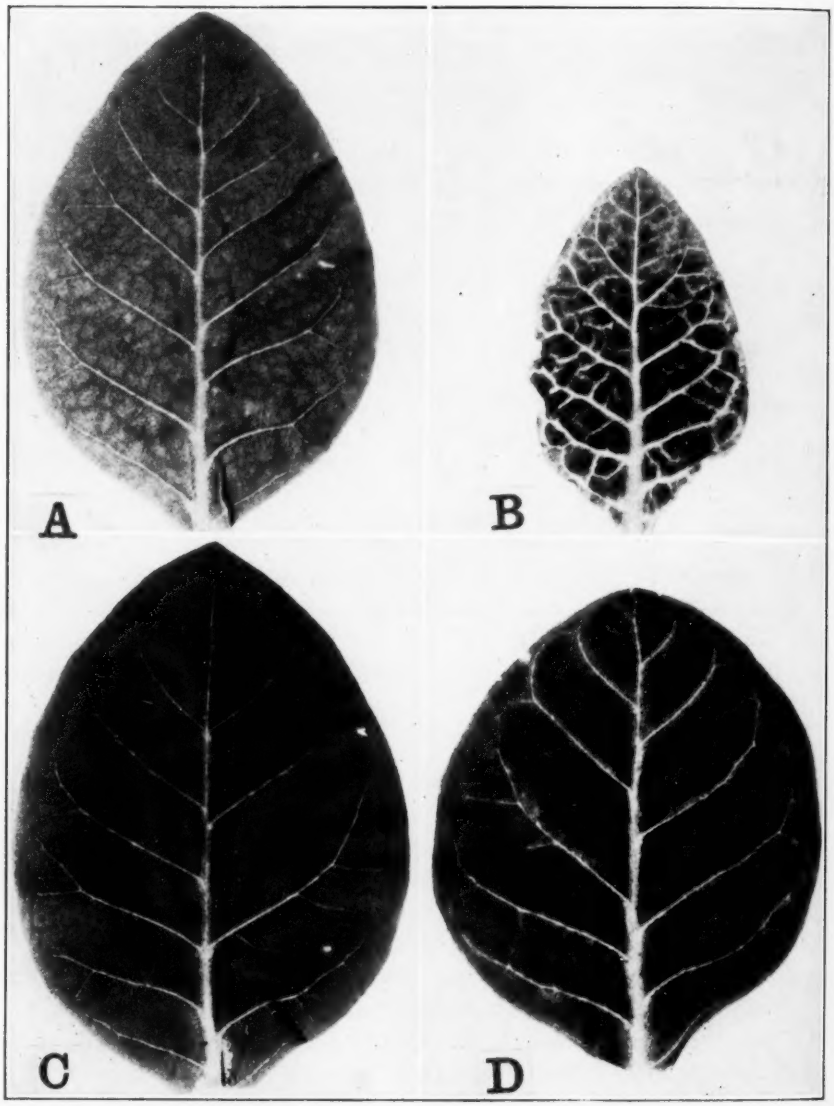

FIG. 2. Comparison of symptoms produced on tobacco by mottle and veinbanding viruses. A. Mottle. B. Spot-necrosis produced by a mixture of the two viruses. C. Control. D. Veinbanding.

These experiments suggested that 2 distinct viruses might be associated with the disease, both of which are readily transmissible by artificial methods, while only one of them is transmitted by aphids, *i.e.*, the veinbanding virus. That the other is the mottle virus (Fig. 2, A), normally present in apparently healthy potatoes, was suggested by Johnson's earlier work on this virus (7, 9).

The veinbanding virus proved readily transmissible from tobacco to tobacco both by aphids and by plant extract (Table 2). That this virus

TABLE 2.—*Summary of results on transmission of the veinbanding virus to tobacco*

Inoculation by	Source of inoculum	No. of plants inoculated	No. of plants showing veinbanding symptoms
Plant extract	Apparently healthy potatoes	80	0
	Veinbanding on tobacco	100	100
Aphids (*Myzus persicae*)	Apparently healthy potatoes	20	0
	Veinbanding on tobacco	17	14

was not present in the apparently healthy potatoes used in the experiments was shown by the fact that it was not recovered from such potatoes by either method of transfer (Table 2). Failure of the aphids to transmit the mottle virus, either from potato or tobacco, is shown in table 3. As shown

TABLE 3.—*Summary of results on transmission of the mottle virus to tobacco*

Inoculation by	Source of inoculum	No. of plants inoculated	No. of plants showing mottle symptoms
Plant extract	Apparently healthy potatoes	80	75
	Mottle on tobacco	75	74
Aphids (*Myzus persicae*)	Apparently healthy potatoes	20	0
	Mottle on tobacco	35	0

in the table, however, this virus may readily be obtained on tobacco free from the veinbanding virus by artificial inoculation of extract from apparently healthy potatoes.

Recombination of the Viruses Causing Rugose Mosaic. Since 2 distinct viruses, which can be maintained separately in tobacco, were obtained from rugose mosaic plants, the next step was to recombine them in tobacco to determine whether spot-necrosis (rugose mosaic) would then develop. Three series of tobacco plants were, therefore, inoculated with different methods of combination of the veinbanding and mottle viruses. As shown in table 4, all tobacco plants inoculated with these mixtures developed spot-

TABLE 4.—*Symptoms developing on tobacco as a result of artificial inoculation with the mottle virus and the veinbanding virus, separately and in combination*

Viruses	No. of plants inoculated	Symptoms		
		Mottle	Veinbanding	Spot-necrosis
Mottle	15	15	0	0
Veinbanding	15	0	15	0
Veinbanding and mottle viruses inoculated separately to each plant	25	0	0	25
Veinbanding and mottle viruses combined before inoculation	20	0	0	20
Veinbanding virus inoculated to plants previously infected with the mottle virus	20	0	0	20
Controls	15	0	0	0

necrosis (Fig. 2, B), while those inoculated with either virus alone did not, thus showing that symptoms of spot-necrosis may be produced by a combination of veinbanding and mottle.

Further evidence that the veinbanding virus is one factor in the production of rugose mosaic of potato is given in table 5. Here it is shown that 25 out of 26 apparently healthy potatoes, which normally contain the mottle virus, developed symptoms of rugose mosaic when inoculated with the veinbanding virus alone. To demonstrate that the mottle virus is the other factor essential for the production of this disease on potato, 25 potato seedlings, which, according to Johnson (7), are mottle-free, were inoculated with the veinbanding virus alone, 8 with a mixture of the mottle and veinbanding viruses, and 3 with plant extract obtained from a rugose-mosaic potato plant. As shown in table 5, the 3 seedings inoculated with rugose mosaic, as well as the 8 inoculated with the mixture of viruses, all developed

TABLE 5.—*Comparative inoculations on apparently healthy potatoes, and on seedling potatoes free from the mottle virus*

Host inoculated	Inoculum	No. of plants inoculated	No. of plants developing rugose mosaic
Apparently healthy potatoes	Veinbanding	26	25
Seedling potatoes	Veinbanding	25	0
	Veinbanding and mottle combined	8	8
	Rugose mosaic	3	3

symptoms of rugose mosaic. The 25 seedlings inoculated with the veinbanding virus alone, however, did not develop symptoms of rugose mosaic; for, although a slight rugosity and mottling of the upper leaves occurred, there was no basal leaf necrosis nor leaf drop. Since the veinbanding virus alone did not cause rugose mosaic in seedling potatoes, it is concluded that the mottle virus, as well as the veinbanding virus, must be present in order to produce typical symptoms of the disease.

The Separation of the Mottle-ring-spot Complex. With the mottle virus in apparently healthy potatoes, there is sometimes found a virus described by Johnson (7) as ring-spot (Fig. 3, C). The question arises as to what part ring-spot plays in the virus complex of spot-necrosis (rugose mosaic). To investigate this relationship it was necessary to obtain ring-spot free from mottle, a condition, it is believed, that has not previously been accomplished.

Following the discovery of 2 viruses in apparently healthy potatoes by Johnson (7) there have been many different names applied to these viruses. Johnson used the names mottle and ring-spot as being descriptive of the symptoms occurring on tobacco, it then not being known whether these viruses existed as such in the potato. It seems quite evident that Valleau and E. M. Johnson (23) included these 2 viruses in what they call "the healthy potato virus"; although, according to their descriptions and illustrations, they obtained symptoms identical with those produced by both mottle and ring-spot. While recognizing these 2 viruses as described by Johnson (7), Burnett and Jones (4) have given them different names, calling one the latent virus and the other the virulent latent virus. In England, Smith (22) describes the symptoms produced by a virus which he calls "ring-spot." This appears to be similar to the potato ring-spot described by Johnson (7). He does not describe a virus similar to mottle, however, and it is not possible from the published descriptions to determine if this

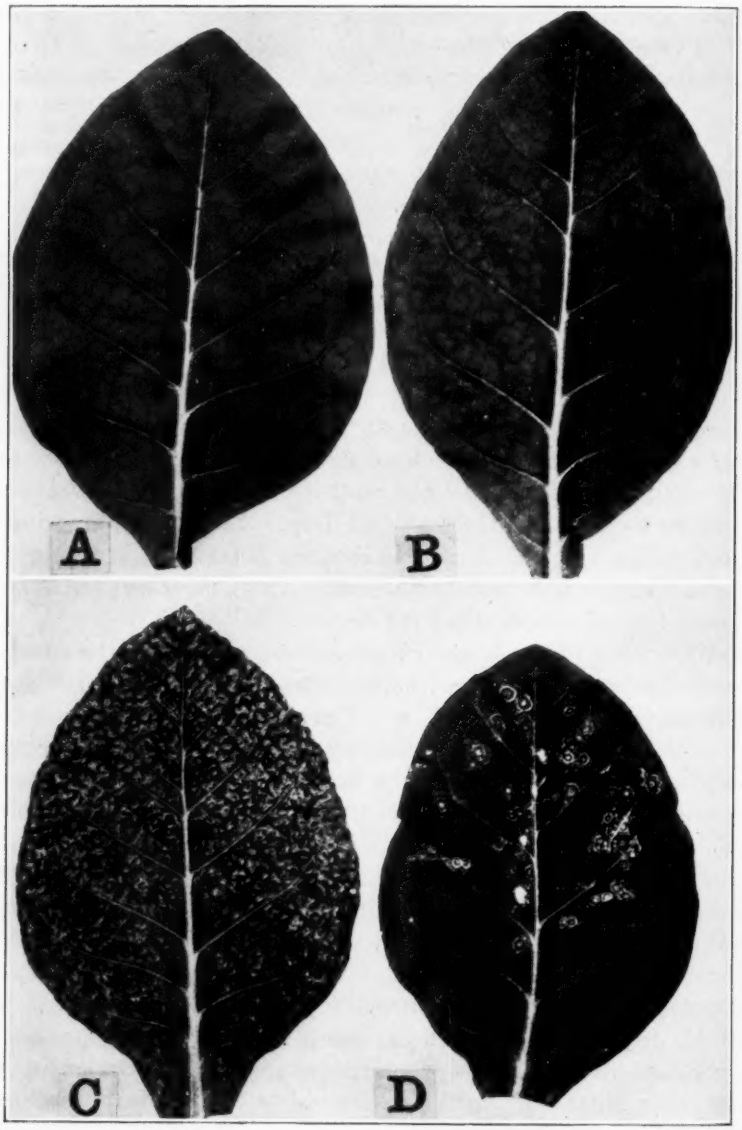

FIG. 3. Comparison of symptoms produced on tobacco by mottle and ring-spot viruses. A. Control. B. Mottle. C. Ring-spot. D. Local lesions produced by ring-spot.

virus was present in combination with his ring-spot. In Holland, Quanjer (16) has worked with a virus that he states is known to be latent in the American varieties Green Mountain, Irish Cobbler, and Rose. Quanjer is

evidently referring to one or both of the viruses found in the apparently healthy American potatoes.

Mention should be made that the ring-spot obtained from apparently healthy potatoes should not be confused with another ring-spot disease of tobacco, first shown by Fromme, Wingard, and Priode (5) to be due to a virus.

Since both the mottle and the ring-spot viruses are found in apparently healthy potatoes, especially in the variety Rural, there has been some question as to their identity as separate viruses. Following his first description of the symptoms produced on tobacco, Johnson (7) expressed the opinion that 2 distinct viruses were probably concerned, although he did not actually separate them on the basis of properties or in any other way. There is little doubt that the mottle virus has often been obtained upon tobacco from apparently healthy potato plants free from the ring-spot virus, since mottle is regularly present, while ring-spot appears to be much less common. So far as can be ascertained, however, there is no indication in the literature that the potato ring-spot virus has been separated from the mottle virus and propagated in a "pure" form.

During the course of the experiments, it was observed that the first symptoms of ring-spot appeared on tobacco about 3 days after inoculation, while the first symptoms of mottle did not appear until about 7 days after inoculation. This suggested a possible difference in the rate of progress of the 2 viruses through the tobacco plant, by means of which their separation might be accomplished. The lowest leaves of young tobacco plants were, therefore, inoculated with a mixture of mottle and ring-spot, and it was found possible by means of inoculations from the tips of these plants at intervals of 6 or 12 hours to obtain what appeared to be pure ring-spot upon tobacco (Table 6). In Series I inoculations were made every 12 hours from the tips of the original plants, the first inoculation being made 12 hours after the introduction of the virus mixture into the plants. Ring-spot was recovered in this series 96 hours after the original inoculation, while mottle was not recovered until after 108 hours. In Series II and III inoculations from the tips of the young leaves were made every 6 hours but were not begun until 66 hours after the introduction of the virus mixture. Ring-spot was first obtained in Series II 72 hours after the original inoculation, although mottle was not recovered until 108 hours had elapsed. Series III did not yield ring-spot until after 84 hours, while mottle was secured in 102 hours.

To determine if the mottle virus was still associated with the ring-spot virus obtained in this manner, property determinations were made. Since, as will be shown later, the ring-spot virus is slightly less resistant to heat, dilution, chemicals, and aging than is the mottle virus, mottle, if

TABLE 6.—*Results of experiments planned to separate the ring-spot and mottle viruses on the basis of their rates of progress through tobacco plants*

Number of hours allowed for viruses to pass through plant	No. of plants inoculated in each series	Series I		Series II		Series III	
		No. of plants showing ring spot	No. of plants showing mottle	No. of plants showing ring spot	No. of plants showing mottle	No. of plants showing ring spot	No. of plants showing mottle
66	5	0	0	0	0	0	0
72	5	0	0	1	0	0	0
78	5	–	–	0	0	0	0
84	5	0	0	3	0	4	0
90	5	–	–	0	0	3	0
96	5	1	0	4	0	5	0
102	5	–	–	1	0	4	2
108	5	4	1?	5	2	5	3
114	5	–	–	5	3	–	–
120	5	4	1	5	5	–	–

present, should appear in tests designed to eliminate ring-spot but not mottle. The ring-spot virus will tolerate dilution of 1 to 10,000 but not 1 to 100,000, and, although mottle will tolerate a dilution of 1 to 100,000, it did not appear at this dilution of the ring-spot virus in any of the trials. Also, in testing the effect of heat on the virus, it was found that after heating at 68° C., a temperature that inactivates ring-spot but not mottle, no mottle developed. In addition, it was found that, although ring-spot is not so resistant to alcohol as is the mottle virus, mottle did not appear where it might be expected to do so if present in the virus extract. From these tests it was concluded that the ring-spot virus obtained in the manner described above was free from the mottle virus and that the 2 viruses are actually distinct entities.

It is particularly worthy of note that a combination of the ring-spot and veinbanding viruses produced symptoms on tobacco very similar to the spot-necrosis produced by a combination of the mottle and veinbanding viruses (Fig. 4). Spot-necrosis symptoms of a severe type were produced in 5 separate trials on a total of 25 tobacco plants by inoculating them with a combination of the ring-spot and the veinbanding viruses. Furthermore, the type of spot-necrosis produced by the ring-spot-veinbanding combination was found to develop necrosis of the veins earlier and to produce a

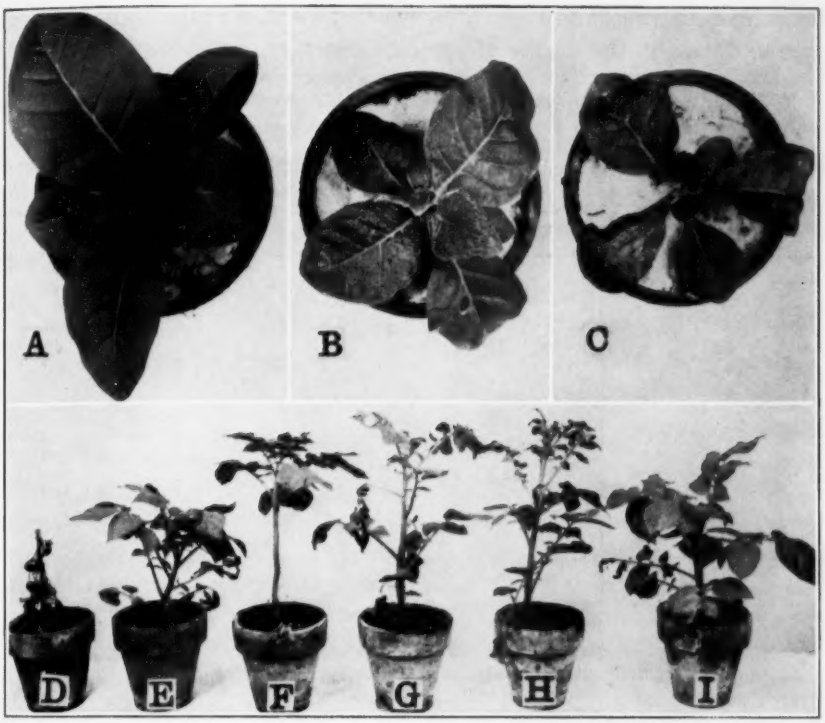

FIG. 4. A–C. Severity of spot-necrosis produced on tobacco by different combinations of viruses. A. Control. B. Combination of veinbanding and mottle viruses. C. Combination of veinbanding and ring-spot viruses. D–I. Symptoms of rugose mosaic produced on 6 common American varieties of potatoes by veinbanding virus. D. Bliss Triumph. E. Early Ohio. F. Green Mountain. G. Irish Cobbler. H. Rural New Yorker. I. Early Rose.

severer type of necrosis than did the mottle-veinbanding combination. Although the 2 viruses, when combined with veinbanding, differ in the severity of spot-necrosis symptoms produced, they are strikingly alike in general behavior. Since, however, the mottle virus is usually present in American varieties of potatoes, while ring-spot is not so common, it is evident that under field conditions the former virus is likely to be the most important in the production of rugose mosaic.

THE MOTTLE VIRUS

The mottle virus used in these experiments has been identified by James Johnson as identical with that described by him in 1925 (7). The presence of this virus in Havana tobacco is indicated by the darker green tissue along the veins, while that between these darkened lines becomes slightly chlor-

otic. Necrotic lines and rings are entirely absent. (Figs. 2, A and 3, B). Some variation in the degree of severity, however, may occur.

TABLE 7.—*A comparison of the thermal death points of the veinbanding, ring-spot, and mottle viruses*

Temperature	Number of plants infected out of 20 inoculated		
	Veinbanding	Ring spot	Mottle
°C.			
50	20		
54	19		
55	17		
56	4		
58	1		
60	0	20	20
65		19	20
68		0	7
70		0	0

TABLE 8.—*The effect of aging upon the infectivity of the veinbanding, ring-spot, and mottle viruses*

Aging of virus	Number of plants infected out of 20 inoculated		
	Veinbanding	Ring spot	Mottle
Days			
None	17	20	20
2	9	20	20
3	8	—	—
4	7	17	20
5	3	—	—
6	0	11	20
8	0	14	19
10		12	19
12		12	20
14		17	20
16		9	18
18		9	14
20		12	15
22		10	13
24		13	13
26		14	10
28		7	9

TABLE 9.—*The effect of dilution upon the infectivity of the veinbanding, ring-spot, and mottle viruses*

Dilution	Number of plants infected out of 20 inoculated		
	Veinbanding	Ring spot	Mottle
None	20	20	20
1–100	20	20	20
1–500	18	—	—
1–1,000	12	19	20
1–5,000	4	—	—
1–10,000	0	12	16
1–100,000	0	0	8
1–1,000,000	—	0	0

TABLE 10.—*The influence of various chemicals upon the infectivity of the veinbanding, ring-spot, and mottle viruses*

Chemical	Strength of chemical	Number of plants infected out of 20 inoculated					
		Veinbanding		Ring spot		Mottle	
		30 min.	60 min.	30 min.	60 min.	30 min.	60 min.
Nitric acid c. p.	1–500	0	0	20	20	20	19
Formaldehyde (37%)	1–100	1	0	16	10	13	10
Alcohol (absolute)	25%	19	16	20	20	20	20
Alcohol (absolute)	50%	1	0	7	6	17	15

The virus is readily transmitted by artificial means, but not by aphids. Failure of aphids to transmit the mottle virus has already been demonstrated in this paper. The mottle virus may readily be obtained on tobacco by artificial inoculation from apparently healthy potatoes of most American varieties or by separation from veinbanding on the basis of its greater resistance to heat and other treatments.

Property studies on the mottle virus show that it will withstand heating, aging, dilution, and chemicals to a greater degree than will either the ring-spot or veinbanding virus (Tables 7–10). This virus is inactivated at a

temperature of 70° C. It will tolerate a dilution of 1 to 100,000, though it is not infectious at a dilution of 1 to 1,000,000. After being subjected to aging for 28 days at room temperature, mottle may be still highly infective. It is only slightly affected by 60-minute exposures to nitric acid (1 to 500), formaldehyde 37 per cent (1 to 100), or 50 per cent alcohol.

THE RING-SPOT VIRUS

The first symptoms of potato ring-spot on Havana tobacco appear 3 to 4 days after inoculation as small local lesions on the inoculated leaves. (Fig. 3, D). These lesions at first consist of a small central necrotic spot around which occur 1 or 2 necrotic rings. Later, the number of rings increases until there may be several in a single lesion. Symptoms on the upper leaves, which appear 6 to 7 days after inoculation, consist of irregular necrotic lines and concentric rings, which often coalesce into large necrotic areas. (Fig. 3, C).

Ring-spot is readily transmitted by artificial means. It does not appear, however, to be transmissible by the aphid *Myzus persicae*, since no infection was obtained in 8 separate trials involving a total of 40 tobacco plants infested with aphids that had previously fed upon ring-spot-infected tobacco plants.

Property studies upon the ring-spot virus show that it is not quite so resistant as the mottle virus, though more so than the veinbanding virus (Tables 7–10). Ring-spot is inactivated at a temperature of 68° C. It is still infectious after aging for 28 days, though its infectivity has apparently been reduced. This virus will tolerate a dilution of 1 to 10,000 but is not infective when diluted 1 to 100,000. Ring-spot is only slightly affected by a 60-minute exposure to nitric acid (1–500) and formaldehyde 37 per cent (1 to 100). It is, however, rendered less infectious by a 60-minute exposure to 50 per cent alcohol.

THE VEINBANDING VIRUS

As the experiments in the University of Wisconsin have been almost entirely confined to the Havana-Seed type of tobacco, a comparison was made of the symptoms produced by veinbanding on this and other distinct types of *Nicotiana tabacum*. The Burley, Orinoco, Turkish, and Pryor types were chosen in addition to Havana Seed for this comparison. The Havana-Seed, Orinoco, Burley, and Pryor types produced symptoms of veinbanding, consisting of a general flattening of the entire plant, a slight clearing of the veins, and a faint chlorosis of the leaves (Fig. 2, D). Although the Turkish variety at first developed symptoms identical with those produced on the other types, a mottling later developed that was very similar to the symptoms caused by the mottle virus obtained from apparently healthy potatoes.

The veinbanding virus is readily transmitted by the aphids *Myzus persicae* and *Macrosiphum solanifolii*, as well as by plant extract. It may be obtained from the rugose mosaic or spot-necrosis complex by means of these insects.

Property studies upon the veinbanding virus show that it is less resistant than either the mottle or the ring-spot virus (Tables 7–10). Veinbanding is inactivated at a temperature of 60° C. It will withstand aging for only 4 or 5 days. This virus will tolerate dilution to 1 to 5,000 but not 1 to 10,000. Veinbanding will not withstand exposure to nitric acid (1 to 500), formaldehyde 37 per cent (1 to 100), or 50 per cent alcohol for 30-minute periods, although it is still infectious after being exposed to 25 per cent alcohol for 60 minutes.

Host Range. To determine a partial host range for the veinbanding virus, several species of solanaceous plants were selected and infested with aphids that had previously fed on rugose-mosaic potato plants. The infestations were made in series of 5 plants each, the juice from all 5 being subsequently combined and inoculated back to tobacco as a test for susceptibility of the host in question. If symptoms of veinbanding appeared upon the latter plants, it was evident that at least 1 plant in the series had been infected.

TABLE 11.—*A test of certain solanaceous plants as possible hosts of the veinbanding virus*

Plants inoculated	No. of series of 5 plants each infested with viruliferous *Myzus persicae*	Symptoms produced	No. of series producing veinbanding upon reinoculation to tobacco
Datura stramonium	4	None	0
Lycopersicon esculentum	6	Vein-clearing	6
Nicandra physaloides	6	None	6
Nicotiana glutinosa	4	Vein-clearing	4
" *rustica*	6	"	6
Physalis pubescens	6	None	6
" *longifolium*	4	"	0
Solanum minuatum	6	"	5
" *melongena*	6	"	0
" *nigrum*	6	"	5
" *carolinense*	4	"	0

It is shown in table 11 that *Lycopersicon esculentum, Nicandra physaloides, Solanum minuatum, S. nigrum, Nicotiana glutinosa, N. rustica,* and *Physalis pubescens* are susceptible to veinbanding since the virus was readily recoverable from infested plants by artificial inoculation to tobacco. On the other hand, no virus was recovered from *Datura stramonium, Physalis longifolium, Solanum melongena,* and *S. carolinense.* Similar results were obtained with *D. stramonium* by Valleau and Johnson (23) and possibly also by K. M. Smith (22), who failed to infect this species with his aphid-transmitted virus. The fact that certain solanaceous plants will act as hosts for veinbanding may be of some practical importance. It seems, as suggested by Valleau and Johnson (23), that certain solanaceous weeds growing near a potato field may possibly become infected with this virus and act as a source of infection for neighboring potato plants that already carry the mottle virus, resulting in the development of rugose mosaic.

Resistance in Potatoes. Johnson (9) has found that a marked difference in susceptibility exists among the common American potato varieties to several potato-virus diseases, including rugose mosaic. As rugose mosaic is now shown to be due to a combination of the veinbanding virus with one or both of the viruses found in the apparently healthy potato, an attempt has been made to determine the degree of resistance exhibited by the common American potato varieties to the veinbanding virus.

The Green Mountain, Bliss Triumph, Early Rose, Early Ohio, Irish Cobbler, and Rural New Yorker varieties were used, 50 plants of each variety being artificially inoculated with the veinbanding virus (Table 12). The results indicate that these varieties may be classed as either susceptible or resistant. The Green Mountain and Bliss Triumph varieties belong in

TABLE 12.—*Relative resistance of certain potato varieties to rugose mosaic when inoculated with the veinbanding virus alone*

Variety tested	No. of plants inoculated	No. of plants infected	Average incubation period in days	Symptoms		
				Necrosis	Mottling	Rugosity
Green Mountain	50	50	17.6	Severe	Good	Severe
Bliss Triumph	50	46	19.4	Very severe	"	Very severe
Early Rose	50	40	30.4	Slight	Slight	Slight
Early Ohio	50	38	29.7	Good	None	None
Irish Cobbler	50	29	28.4	"	"	"
Rural New Yorker	50	21	28.8	Slight	Good	Good

the susceptible class, since, in both, the disease has a relatively short incubation period, high percentages of infection are secured, and severe symptoms of rugose mosaic develop (Fig. 4, D–I). Green Mountain is probably slightly more susceptible than Bliss Triumph, since a higher percentage of infection was obtained on the former and the incubation period was somewhat shorter, although the symptoms are more severe in Bliss Triumph. None of the Green Mountain potatoes were entirely destroyed by this disease, while about 25 per cent of the Bliss Triumph potatoes were killed.

The Early Rose, Early Ohio, Irish Cobbler, and Rural New Yorker varieties evidently belong in the resistant group (Fig. 4, D–I), with Early Rose the least resistant and Rural New Yorker the most resistant of the 4. While the incubation periods of the disease in these 4 varieties are almost the same, the varieties vary considerably in the percentage of infection obtained, the Rural variety yielding about half as many infected plants as does Early Rose, and Irish Cobbler about 75 per cent as many.

From the experiments carried on by Johnson (9) and by the writer it appears there is a decided variation in the resistance exhibited by the common American potato varieties to the veinbanding virus, suggesting possibilities for future control measures.

COMPARATIVE STUDIES WITH VIRUSES FROM FOREIGN SOURCES

One of the most important problems in connection with potato-virus diseases is the correlation of research in various countries with reference to the specific identity of the viruses studied. In most cases it has remained almost impossible on the basis of symptomatology definitely to identify a virus disease, much less a virus, and until this is done much uncertainty will exist in the literature. For the present an exchange of materials for study appears to offer the most promising line of attack. Through the courtesy of R. N. Salaman, H. M. Quanjer, E. S. Schultz, D. Folsom, and others, the Wisconsin laboratories have been able to secure various viruses for comparative study, in a preliminary way, with the rugose-mosaic viruses. It may be said that our rugose mosaic agrees with Schultz and Folsom's rugose mosaic, which, in turn, is presumed to be identical with potato crinkle in Europe, as described by Murphy (11) and by Quanjer (14).

Salaman (17), in England, states that a disease named by him "Crinkle 'A'" is due to a combination of viruses, one element of which is responsible for the crinkle-mosaic type of symptom and another for the streak type. Since he also considers potato streak to be the same as rugose mosaic, the two component viruses of rugose mosaic might be expected to be comparable with those concerned in the production of streak.

A study has been made upon Salaman's Crinkle A as occurring in the potato variety President. Tobacco plants, artificially inoculated with extract from President potatoes affected with Crinkle A developed a mottling that appeared similar to that produced by the mottle virus. When the veinbanding virus was introduced into tobacco plants containing this virus spot-necrosis resulted, indicating further that Crinkle A includes the mottle virus. That Crinkle A did not include the veinbanding virus was inferred from the fact that artificial inoculation to tobacco yielded symptoms of mottle only and not of spot-necrosis.

These results suggested the possibility that those potato varieties in which Crinkle A caused streak might contain the veinbanding virus, particularly if, according to Salaman, streak and rugose mosaic are identical. Three varieties of potato in which Crinkle A was said to produce streak and 3 in which it was said to produce crinkle mosaic were tested for the presence of both the veinbanding and the mottle viruses. This was accomplished by means of direct inoculation from potato to tobacco. Extract from these potatoes was combined also with the veinbanding virus in a further test of the presence or absence of mottle with the expectation that, if this virus were present, spot-necrosis would be produced. Similarly, another portion of extract was combined with the mottle virus and inoculated to tobacco to determine whether veinbanding were present.

The varieties Up-to-Date, Duke of York, and King Edward, which, according to Salaman (17), belong to the streak-producing group, showed no evidence of containing the veinbanding virus, while the presence of mottle virus was demonstrated only in the Duke of York variety. The varieties Arran Victory, President, and Abundance, said to develop crinkle mosaic following inoculation with Crinkle A, were shown to contain neither veinbanding nor mottle. From these results it, therefore, seems unlikely that Salaman's streak is identical with our rugose mosaic.

Preliminary studies made upon certain potato-virus diseases obtained from Holland from H. M. Quanjer indicate that some of these diseases may be caused, in part at least, by viruses similar to those associated with our rugose mosaic. The presence of the veinbanding virus in potato plants that, according to Quanjer, were infected with "acropetal necrosis" (16) was demonstrated by artificial inoculation of extract to tobacco, symptoms of veinbanding developing upon the inoculated tobacco plants. Additional evidence of the presence of the veinbanding but not the mottle virus in these potatoes was obtained by combining part of the extracted juice with the veinbanding virus and part with the mottle virus and subsequently inoculating each combination to a series of tobacco plants. Tobacco plants inoculated with the veinbanding combination developed symptoms of veinbanding only, indicating the absence of mottle from the original potato

plants, while plants inoculated with the mottle combination developed symptoms of spot-necrosis, demonstrating the presence of the veinbanding virus in the potatoes. This fact was confirmed by inoculation of Bliss Triumph potatoes, carrying mottle, with the extract from the original potato plants, which resulted in symptoms of severe rugose mosaic.

A series of potatoes that, according to Quanjer, were infected with "acronecrosis" were tested in a similar manner and were also found to contain the veinbanding but not the mottle virus. The mottle virus, on the other hand, was recovered from potatoes said by Quanjer to be infected with leaf roll. This virus, strangely enough, was not recovered from potatoes said to be carriers of the apparently healthy potato virus.

From these preliminary studies it may be concluded that the veinbanding and mottle viruses are both present in England and Holland, though the question of their relative importance in the field remains obscure.

DISCUSSION

The potato-virus-disease problem, as a whole, is greatly complicated by the presence of one or more viruses in apparently healthy American potatoes. The problem may be said to be complicated still further by the irregular association of these viruses with apparently healthy European stocks of potatoes. Nevertheless, as far as rugose mosaic is concerned, it is evident that the information that has been accumulating from several sources in recent years is serving to clarify a situation that is much more complex than had been realized. The results, as a whole, go to show that the symptom complex, known as rugose mosaic in America, must generally be due to the mottle and the veinbanding viruses acting in combination. It is quite as evident, however, that an almost identical symptom expression may result from the ring-spot and veinbanding complex either alone or in combination with the mottle virus. This evidence would suggest rather than preclude the possibility of still other viruses being concerned in similar expressions of disease. It is believed, however, that when potato diseases of the rugose-mosaic, crinkle, and streak types have been adequately studied in various parts of the world the causal agencies will be found to have much in common. Such studies must evidently be based particularly upon the isolation of the viruses concerned and upon determinations of their comparative properties, modes of transmission, host range, and other distinguishing characteristics rather than solely upon extensive descriptions of symptoms.

The increased knowledge concerning the nature of the rugose-mosaic disease suggests several possible lines of endeavor in the direction of control. Fortunately, when the viruses concerned are combined, the result is a disease that tends to be self-exterminating, even though the viruses by them-

selves are admirably adapted to self-perpetuation. The prevention of insect dissemination of a virus disease through insect control is not yet a promising line of control in the field. The elimination of the insect-transmitted virus through tuber indexing of the potato stock itself, seems most likely to succeed. Should it develop that other host plants actually harbor the veinbanding virus to any extent, this would no doubt offer added but perhaps not insurmountable difficulties to the propagation of virus-free stocks. Still another line of control is well worthy of careful consideration in districts where rugose mosaic is a serious disease. Manifestly, the elimination of the mottle virus, normally present in apparently healthy potatoes, would prevent the development of the more serious rugose-mosaic disease, even though veinbanding were prevalent. Since potatoes free from the mottle virus can apparently be found (4) and since this virus is not aphid-transmitted, it would seem to be only a routine matter to build up large stocks of seed potatoes of different varieties free from this constituent of the disease.

Lastly, the probability of utilizing existing varieties of resistant potatoes or of developing new strains resistant to one or more of the viruses concerned in rugose mosaic should not be overlooked.

SUMMARY

Further evidence is presented to show that the rugose-mosaic disease of potato is due to the combined effect of the mottle virus, normally present in apparently healthy potatoes, with the veinbanding virus. The potato ring-spot virus may, however, replace, with almost identical results, the mottle virus in the combination.

Both the mottle virus and the ring-spot virus are easily transmitted by plant extract, but all attempts to transmit these by means of aphids have failed. The veinbanding virus, which is also transmissible by plant extract, is readily transmitted as well by the aphids *Myzus persicae* and *Macrosiphum solanifolii*.

The differential behavior of these viruses with respect to insect transmission serves as a most simple means of isolating the veinbanding virus from the rugose-mosaic complex. The mottle virus, on the other hand, may be readily separated from the complex on the basis of differential properties.

The mottle virus, as secured either from the rugose-mosaic complex or from apparently healthy potatoes, may sometimes be combined with the potato ring-spot virus, while the latter has not yet been found except in association with mottle.

Mottle and ring-spot apparently can not be separated upon the basis of transmission or differential properties. It was found, however, that the

ring-spot virus could be isolated from mottle by utilizing the differential rate of progress of the 2 viruses through the tobacco plant.

The thermal death point of the veinbanding virus was found to be 60° C., that of ring-spot 68° C., and that of mottle 70° C. The veinbanding virus resists aging *in vitro* only about 5 days, whereas ring-spot and mottle may resist aging as long as 28 days. Veinbanding will withstand a dilution of only about 1 to 5,000, and ring-spot 1 to 10,000, whereas mottle will withstand a dilution as high as 1 to 100,000. Veinbanding is also more sensitive to chemical treatments than is ring-spot or mottle.

Tests on resistance to rugose mosaic in 6 common American varieties of potato indicate that this varies with the variety and, in the Rural New Yorker, at least, it is perhaps great enough to warrant use of this variety as a resistant potato.

Certain solanaceous plants are shown to be susceptible to the veinbanding virus. This suggests the possibility of aphids transmitting veinbanding from weed hosts to neighboring potato plants, which, since they already harbor the mottle virus, would then develop rugose mosaic.

Comparative studies with virus diseases from foreign sources indicate that viruses similar to or identical with mottle and veinbanding may be found in foreign potatoes. The existence of certain varieties or strains free from the mottle and ring-spot viruses suggests the use of such potato stocks as a possible method of control of rugose mosaic.

UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN.

LITERATURE CITED

1. ALLARD, H. A. Effects of various salts, acids, germicides, etc., upon the infectivity of the virus causing the mosaic disease of tobacco. Jour. Agr. Res. **13**: 619–637. 1918.
2. APPEL, O. Leaf-roll diseases of the potato. Phytopath. **5**: 139–148. 1915.
3. ATANASOFF, D. A study into the literature on stipple-streak and related diseases of the potato. Meded. Landbouwhoogesch. [Wageningen], vol. **26**, no. **1**. 52 pp. 1922.
4. BURNETT, GROVER, and L. K. JONES. The effect of certain potato and tobacco viruses on tomato plants. Wash. Agr. Exp. Sta. Bul. 259. 1931.
5. FROMME, F. D., S. A. WINGARD, and C. N. PRIODE. Ringspot of tobacco; an infectious disease of unknown cause. Phytopath. **17**: 321–328. 1927.
6. HOGGAN, ISMÉ A. The peach aphid (*Myzus persicae* Sulz.) as an agent in virus transmission. Phytopath. **19**: 109–123. 1929.
7. JOHNSON, JAMES. Transmission of viruses from apparently healthy potatoes. Wis. Agr. Exp. Sta. Res. Bul. 63. 1925.
8. ————. The classification of plant viruses. Wis. Agr. Exp. Sta. Res. Bul. 76. 1927.

9. ————. The classification of certain virus diseases of the potato. Wis. Agr. Exp. Sta. Res. Bul. 87. 1929.
10. KOCH, KARL. The potato rugose mosaic complex. Science, n. s. **73**: 615. 1931.
11. MURPHY, P. A. Investigations of potato diseases. Canada Exp. Farms Div. Bot. Bul., II, 44. 1921.
12. ————. Investigations on the leaf-roll and mosaic diseases of the potato. Dept. Agr. and Tech. Instr. Ireland Jour. **23**: 20–34. 1923.
13. ORTON, W. A. Potato wilt, leaf-roll, and related diseases. U. S. Dept. Agr. Bul. 64. 1914.
14. QUANJER, H. M. New work on leaf-roll and allied diseases in Holland. Rpt. Internat. Potato Conf. London **1921**: 117–145. 1921.
15. ————. General remarks on potato diseases of the curl type. Rpt. Internat. Conf. Phytopath. and Econ. Ent. Holland **1923**: 23–28. 1923.
16. ————. The methods of classification of the plant viruses, and an attempt to classify and name potato viroses. Phytopath. **21**: 577–613. 1931.
17. SALAMAN, R. N. Crinkle "A," an infectious disease of the potato. Proc. Royal Soc., Ser. B, **106**: 50–83. 1930.
18. ————, and R. H. LE PELLEY. Para-crinkle: a potato disease of the virus group. Proc. Royal Soc., Ser. B, **106**: 140–174. 1930.
19. SCHULTZ, E. S., and D. FOLSOM. Transmission, variation, and control of certain degeneration diseases of Irish potatoes. Jour. Agr. Res. **25**: 43–118. 1923.
20. ————, ————, F. M. HILDEBRANDT, and L. A. HAWKINS. Investigations on the mosaic disease of the Irish potato. Jour. Agr. Res. **17**: 247–274. 1919.
21. SMITH, KENNETH M. Studies on potato virus diseases. VI. Further experiments with the virus of a potato mosaic upon the tobacco plant. Ann. Appl. Biol. **16**: 382–399. 1929.
22. ————. Composite nature of certain potato viruses of the mosaic group. Nature **127**: 702. 1931.
23. VALLEAU, W. D., and E. M. JOHNSON. The relation of some tobacco viruses to potato degeneration. Ky. Agr. Exp. Sta. Bul. 309. 1930.

PHYSIOLOGIC FORMS OF WHEAT STEM RUST IN KANSAS AND NEBRASKA[1]

GEORGE L. PELTIER[2]

(Accepted for publication June 28, 1932)

INTRODUCTION

The writer here presents a summary of an intensive study of the primary sources of wheat stem rust in Kansas and Nebraska, based on observations of the spread of rust and on the annual occurrence of the physiologic forms of *Puccinia graminis tritici* (Pers.) Eriks. and Henn. during a 3-year period (1928–30). The methods employed in making the surveys and collecting the material for the identification of the forms were essentially the same as those already described in detail by Peltier and Thiel (3).

Since the publication of this paper Stakman, Levine, and Wallace (5) and Wallace (6) have reported the physiologic forms identified by them in the United States and Mexico, but particularly those occurring in the Mississippi Valley over a 3-year period (1926–28). Recently, Newton, Johnson, and Brown (1) have summarized the physiologic forms of wheat rust found in Canada over an 11-year period (1919–29). Peltier (2), on the basis of 6 years' observations, has discussed some of the aspects of the spread of stem rust in Nebraska.

PHYSIOLOGIC FORMS FOUND IN 1928

Near Barberries. In Kansas, aeciospores were first noted the last week in May in Republic, Saline, and Riley counties. Uredinia were first observed on grasses near infected bushes on June 6, 13, and 14, respectively. At this time only a light infection on grasses was found. Collections on grasses and grains near infected barberries in Kansas all proved to be *P. graminis secalis* (Pers.) Eriks. and Henn.

In Nebraska, uredinia on grasses and grains near barberries were first noted on June 10, 14, and 15, in Johnson, Merrick, and Polk counties and later in the season in Merrick, Hall, York, and Saunders counties. The

[1] Paper No. 119 of the Journal Series of the Nebraska Agricultural Experiment Station. Cooperative investigations between the Department of Plant Pathology, Nebraska Agricultural Experiment Station, and the Division of Barberry Eradication, Bureau of Plant Industry, United States Department of Agriculture.

[2] The writer wishes to acknowledge his indebtedness to Dr. C. R. Ball, formerly of the Division of Cereal Crops and Diseases, to Mr. F. C. Meier, Division of Barberry Eradication, Bureau of Plant Industry, United States Department of Agriculture, and to Mr. H. H. Foster and Mr. Floyd Schroeder, former graduate assistants in the Department of Plant Pathology, Nebraska Agricultural Experiment Station.

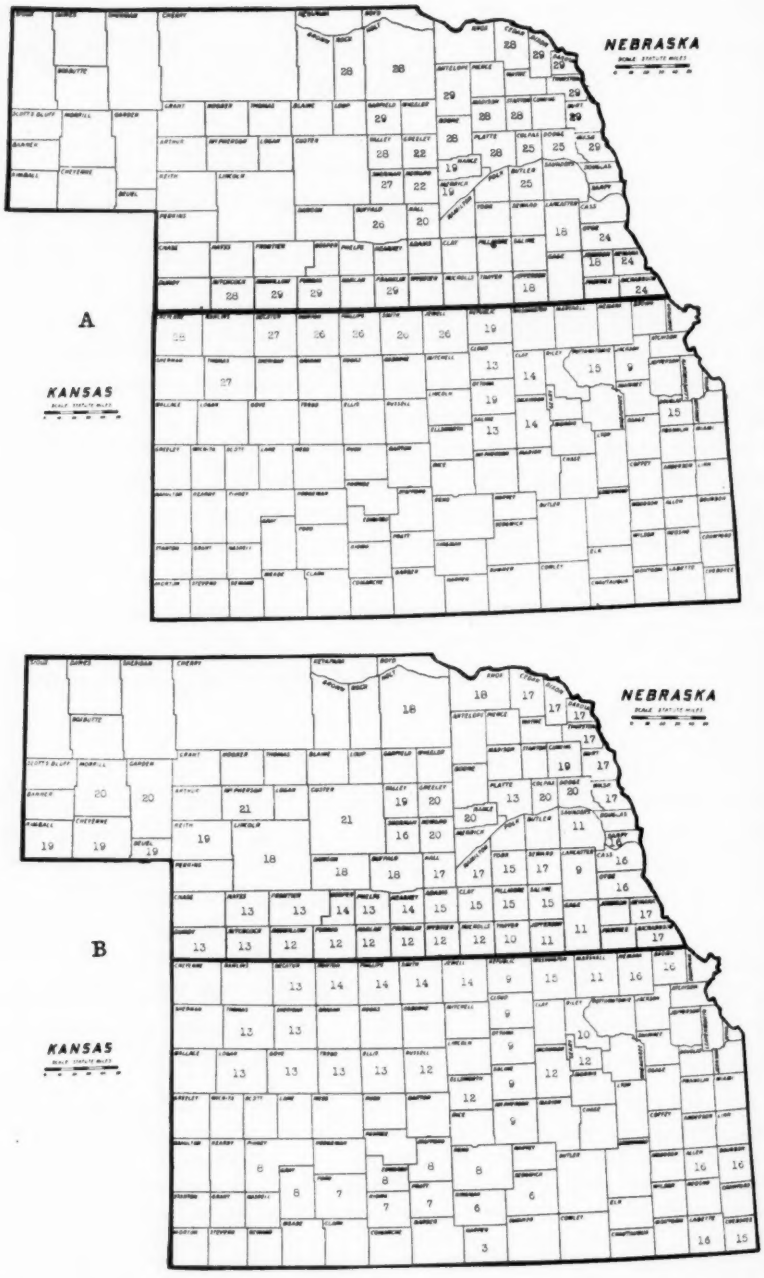

FIG. 1. Date in June when primary uredinia, not directly traceable to barberries, were collected in various counties in Kansas and Nebraska. A. 1928. B. 1929.

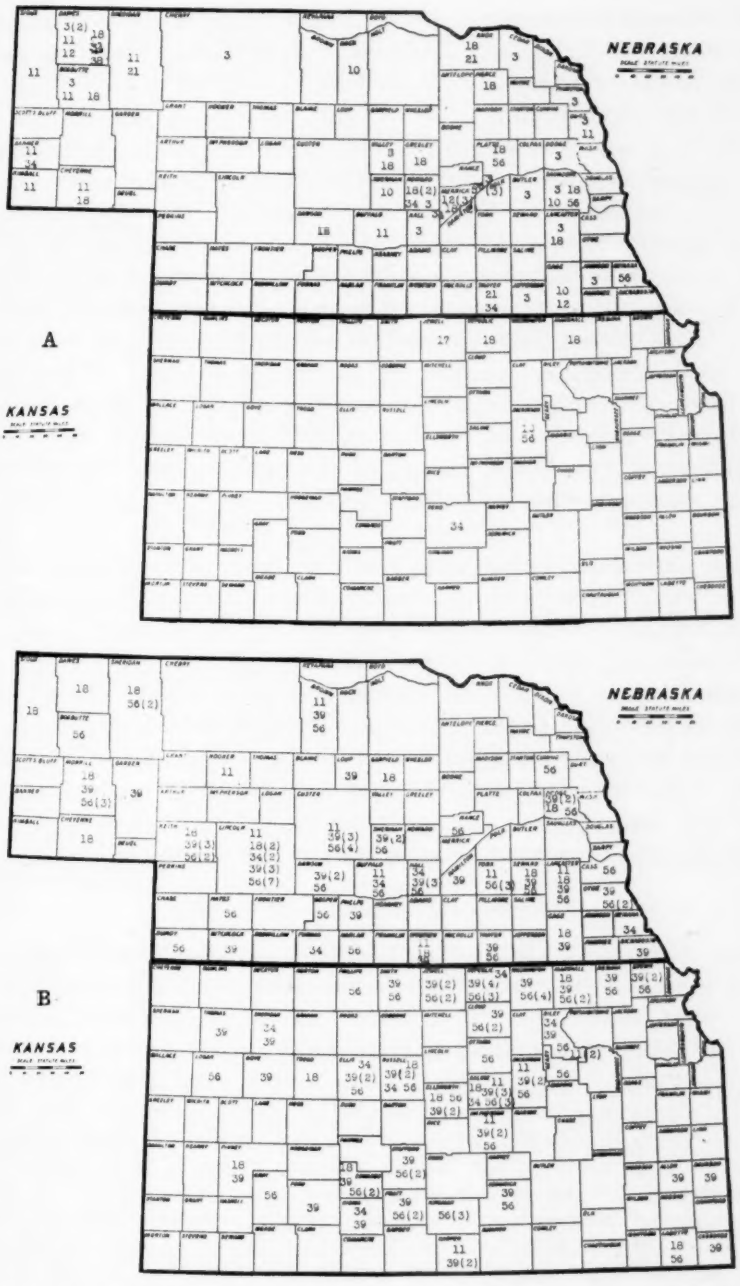

FIG. 2. Distribution of the physiologic forms of wheat stem rust collected in Kansas and Nebraska. A. 1928. B. 1929.

wheat stem-rust forms were identified from collections obtained near 5 barberry locations (Table 1). Physiologic form 3 was found in Johnson County (June 10), forms 12, 13, and 34, in Merrick County (June 28 to July 3), and form 56, in Saunders County (July 2). The uredinia found near infected bushes in Johnson County were the first to be discovered in Nebraska and antedate by 1 week the finding of uredinia not directly traceable to barberries.

Not Directly Traceable to Barberries. In northern Kansas, the first primary uredinia were found the second week in June. Primary uredinia were not observed in northwestern Kansas until the last week in June (Fig. 1, A). In Nebraska, primary uredinia were first observed on winter wheat June 18, in Jefferson, Johnson, and Lancaster counties. The following week a trace of stem rust could be found over most of the State. Stem rust developed very slowly and few secondary and succeeding generations of urediniospores were found. Owing to the fact that stem rust was late in making its appearance, wheat was harvested without any estimable loss from this rust.

Five physiologic forms were identified from the collections made in Kansas, while 9 forms were obtained from the collections made in 56 counties in Nebraska (Table 1 and Fig. 2, A). Of the 9 forms found in Nebraska only 2, forms 3 and 18, were collected throughout the entire season. Apparently these forms were the dominant and prevailing ones in Nebraska, as they were found in all parts of the State. Form 11 seemed to have been generally distributed over the wheat section of western Nebraska. The other physiologic forms were found in small amounts but fairly well scattered over eastern Nebraska. With the exception of form 17, all the others present in Kansas were found also in Nebraska.

PHYSIOLOGIC FORMS FOUND IN 1929

Near Barberries. In Kansas, a total of 11 properties with barberries present was observed in some detail for a period of several weeks in 5 counties. Notes made on several of these locations are presented in order to show the rate of development of rust on the near-by grains and grasses.

Riley County. Hedge with a trace to light infection. Aecia mature May 7. Occasional uredinia first noted on *Agropyron smithii* May 30. On June 8 a trace of infection was found on *A. smithii* 75 ft. and on *Hordeum pusillum* 20 ft. north of the hedge; whereas, 10 to 15 ft. from the hedge, a prevalence of 5 per cent infection was noted. About 10 ft. to the east of the hedge *A. smithii* had only a trace of rust. No rust was found to the west and south of the hedge. Aeciospores infected wheat and physiologic form 34 was later obtained. A collection of uredinia on *H. pusillum* proved to be the rye stem rust.

Saline County. Hedge with a trace of infection. Aecia mature May 9. Occasional uredinia noted on *Agropyron smithii* and *Hordeum pusil-*

TABLE 1.—*Annual occurrence of physiologic forms of Puccinia graminis tritici in Kansas and Nebraska*

Physiologic form No.	Number of times collected									
	Uredinia near barberry		Primary uredinia		Secondary and succeeding uredinia		Fall uredinia		Total	
	Kans.[a]	Nebr.[b]	Kans.	Nebr.	Kans.	Nebr.	Kans.	Nebr.	Kans.	Nebr.
1928										
3		1		4		8		4		17
10				2	1	2			1	4
11						8		1		9
12		2				3				5
17					1				1	
18		1		4	2	8		2	2	15
21						2		1		3
34		1		1	1	3			1	5
38						1				1
56		1		2	1	3			1	6
Total number of forms									5	9
Number of collections									6	65
1929										
11			5	3	1	5			6	8
18			7	7	1	7			8	14
34	1		6	4	1	2			8	6
39		3	41	24	1	8			42	35
56			35	29	6	11			41	40
Total number of forms									5	5
Number of collections									105	103
1930										
15			11	2					11	2
34			11			10			11	10
36	2		39	11		37			41	48
38			8	2		8			8	10
49			1			2			1	2
56				1		2				3
Total number of forms									5	6
Number of collections									72	75

[a] 1928. Collections from 5 properties near infected bushes in 3 northern counties were identified as *P. graminis secalis*.

1929. Collections from 11 properties near infected bushes in 5 northern counties in 8 instances were identified as *P. graminis secalis*.

1930. Collections from 7 properties near infected bushes in 2 northern counties in 5 instances were identified as *P. graminis secalis*.

[b] 1930. Collections from 2 properties near infected bushes in Greeley County were identified as *P. graminis secalis*.

lum May 29. On June 5 scattering infections were found 60 to 65 ft. west of the hedge on *A. smithii* and only 18 ft. on *H. pusillum*. About a 3 per cent prevalence was present on these 2 grasses within 10 ft. of the bushes. South of the hedge rust extended about 50 ft. on *A. smithii* with a 2 per cent prevalence within 10 ft. of the hedge. Very little change in the prevalence and severity of rust was noted on June 25. Collections from infected barberry leaves and grasses all yielded the rye stem rust.

Republic County. Hedge with a heavy infection. Aecia mature May 10. Occasional uredinia noted on *Agropyron smithii* and *Hordeum pusillum* on May 27. By June 4 a light infection had spread 25 ft. west of the hedge on *H. pusillum* and only 15 ft. on *A. smithii*. Within 10 ft. of the hedge a 25 per cent severity was found on both grasses, while farther away the percentage of infection dropped rapidly to less than 1 per cent. East of the hedge rust could be noted for 90 ft. on *H. pusillum* and only 25 ft. on *A. smithii,* while a 25 per cent infection occurred within 10 ft. of the hedge on *H. pusillum* and only 5 to 10 per cent on *A. smithii*. To the south rust extended about 50 ft. on both grasses. North of the hedge *H. jubatum* had 2 to 3 per cent infection 200 to 275 ft. away. Within 100 ft. 3 to 5 per cent infection occurred. Within 50 ft. of the bushes the percentage of infection in *H. pusillum* increased to 15 per cent, and within 25 ft. to 25 per cent. By June 15 the rust had spread 160 ft. to the west and east and 750 ft. to the north. The percentage of rust varied from 2 to 25 per cent near the bushes. No additional spread or increase in the severity of rust was noted after this date. Collection from infected barberry leaves and grasses all yielded the rye stem rust.

Smith County. Two bushes with a light infection. Aecia mature May 11. On May 31 an occasional pustule was found 15 to 20 ft. from the bushes on *Hordeum pusillum* and *H. jubatum*. By June 14 a 5 to 10 per cent infection was present on *H. pusillum* within a 10-ft. radius of the bushes. A trace of infection occurred on *H. pusillum* and *Hystrix patula* up to 60 ft. north of the bushes. *Hordeum jubatum* had a 50 to 60 per cent infection within 15 ft. of the bushes, while only a trace could be found on *Agropyron smithii*. Collections made from infected grasses all yielded the rye stem rust.

The above notes serve to give an idea of the development and rate of spread of stem rust from infected barberry bushes. At 8 such locations in Riley, Saline, Republic, and Smith counties rye stem rust was found near barberries, while wheat stem rust occurred only on 1 property with infected bushes. (Table 1). Uredinial collections on grasses from near 2 locations failed to infect wheat, oats, or rye.

In Nebraska, only 1 property with infected barberries was found rather late in June. Collections made on *Hordeum jubatum* near the bushes and from barley $\frac{1}{8}$ and $\frac{1}{4}$ mile away all yielded the wheat stem rust, which was later identified as physiologic form 39. (Table 1).

Not Directly Traceable to Barberries. The first primary uredinia were collected June 3 in Harper County, Kansas. A few days later primary uredinia were found in a number of the southern counties and were, for

the most part, fully developed and easy to find. In the western part of the State only a light primary infection occurred and rust was harder to find. In figure 1, B the date in June that primary uredinia were collected in the various counties is indicated.

The first uredinia not traceable to barberries were found in Lancaster County, Nebraska, June 9. During the same week a trace of rust was found across the whole southern part of the State. By the third week in June stem rust could be found in small amounts in all parts of Nebraska. Because of hot, dry winds, during the last 2 weeks in June, however, secondary infections developed slowly. While there was a fairly well-scattered primary infection throughout both States early enough to have produced a rust epidemic, the weather conditions were such that the wheat matured rapidly under the influence of the hot, dry winds, conditions extremely unfavorable for the further development of stem rust. Thus, there was no estimable loss from wheat stem rust in Kansas and Nebraska in 1929.

From the collections in Kansas representing primary uredinia, 5 physiologic forms were identified. (Table 1 and Fig. 2, B). The same 5 forms also were obtained from the collections of secondary and succeeding uredinia. It is of interest to note that all collections of infected barley yielded negative results when placed on wheat. It is apparent that forms 39 and 56 were the dominant and prevalent ones in Kansas. In many instances they were isolated from the same collection, so that they were closely associated throughout the State. The other 3 forms were found fairly well distributed but in smaller amount over most of Kansas.

The same 5 forms found in Kansas also were identified from the collections representing primary, secondary, and succeeding uredinia in Nebraska. The number of times the forms were found and their distribution are given in table 1 and figure 2, B. Here, again, it will be noted that forms 39 and 56 were the dominant and prevailing ones and were associated many times as mixtures in the same collection. The other 3 physiologic forms were fairly well distributed in smaller numbers over most of Nebraska.

A number of rust-infected wheat plants were sent the writer from points in Texas and Oklahoma, and form 39 was consistently isolated from these collections.

PHYSIOLOGIC FORMS FOUND IN 1930

Near Barberries. A total of 7 properties in 3 counties in northern Kansas, with barberries present, was observed the middle of June. Aecia were found on most of the bushes, with only a trace to a light infection, and from an occasional to few aecia per leaf. All but 2 collections of infected grasses near these bushes yielded rye stem rust. Two collections of wheat from near an infected barberry were identified as form 36 (Table 1). A rather heavy

infection occurred on winter wheat adjacent to these bushes on June 19, the severity varying from 25 to 50 per cent near the bushes to a trace to 25 per cent 70 ft. away.

Only 2 instances of spread of rust from infected barberries were located in Nebraska. On July 5, scouts found 2 properties in Greeley County, where bushes were moderately infected. All collections of grasses and grains near the bushes and for a mile away yielded rye stem rust.

Not Directly Traceable to Barberries. The first uredinia in Kansas were collected on May 29 in Harper County, but it was somewhat later before uredinia could be readily found. Between June 9 and 24 over 100 lots representing primary uredinia were collected in 54 counties of Kansas (Fig. 3, A). While stem rust was found in all sections of the State the second week of June, it was not a heavy primary infection. The wheat matured rapidly and the yields for the most part were high. No measurable loss from stem rust occurred in Kansas.

The first uredinia not directly traceable to barberries were found on June 8 in Jefferson and Thayer counties, Nebraska. During the same week primary uredinia were found across the whole southern half of the State. By the end of the third week in June primary uredinia occurred in small amounts in all parts of Nebraska (Fig. 3, A). Some secondary and succeeding generations of uredinia were collected, but, owing to the rapidity with which the grain matured, no estimable loss from stem rust occurred. Over 200 collections in 76 counties were obtained in the State in 1930, some of which were lost when the rust was transferred to wheat in the greenhouse.

Through the cooperation of workers in several Southern States, collections of wheat stem rust were obtained from Denton, San Antonio, Dalhart, Big Springs, and Canyon, Texas. From this material form 36 was consistently obtained. Likewise, material from Stillwater, Lawton, Woodward, and Norman, Oklahoma, yielded form 36, together with form 38. From a few collections made in Missouri and Arkansas form 38 was obtained.

Five physiologic forms were identified from the collections made in Kansas (Table 1). It is apparent from figure 3, B that form 36 was the dominant and prevalent one, present as primary uredinia in Kansas. Physiologic forms 15, 34, and 38, while found in smaller numbers, were fairly well distributed over the State. Only one collection of form 49 was found in southeast Kansas.

The same 5 physiologic forms found in Kansas also were present in Nebraska. A sixth form was identified from 3 lots of material widely scattered over the southern part of the State (Table 1). Again, it will be noted that form 36 was the prevailing and dominant form in Nebraska. Forms 34 and 38 were fairly abundant and widely scattered. Only an occasional collection of the other 2 physiologic forms was found.

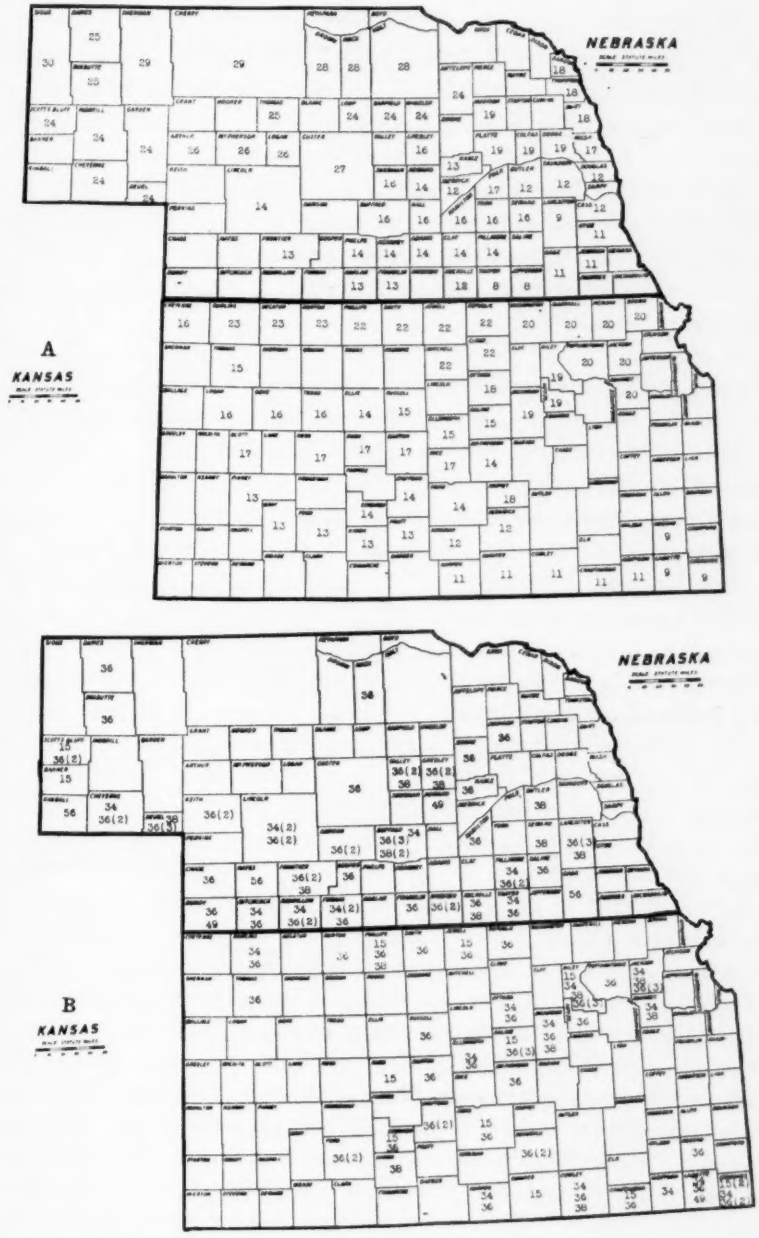

FIG. 3. A. Date in June, 1930, when primary uredinia, not directly traceable to barberries, were collected in various counties in Kansas and Nebraska. B. Distribution of physiologic forms of wheat stem rust collected in Kansas and Nebraska in 1930.

DISCUSSION

Of the 11 localities with infected barberry bushes present, under observation in Kansas since 1925 (3), in only 2 instances has wheat stem rust been found on near-by infected grasses and grains. In nearly every case the rye stem rust has been present. The same bushes visited over a 5-year period in Republic County have consistantly yielded rye stem rust.

With the continued eradication of barberries in Nebraska, it becomes more difficult to find infected barberries spreading rust early in the season. The spread of wheat stem rust from barberries is not so much a factor as it was in former years.

Primary uredinia not directly traceable to barberries appear usually in small amounts, but widely distributed in Kansas, about the first of June, and usually by the end of the same week in southern Nebraska. In occasional years, as in 1928, primary uredinia appear somewhat later. In both western Kansas and Nebraska uredinia are found from 1 to 2 weeks later.

The winter wheats commonly grown in Kansas are Black Hull, Kanred, and Turkey, and in Nebraska, Nebraska No. 60, Kanred, and Turkey. Black Hull, Turkey, and Nebraska No. 60 have proved susceptible to all the physiologic forms found in these 2 States. Kanred, however, is resistant to a number of the forms. It has been noted also that fields of beardless wheat, found occasionally in this area, usually are more heavily infected than adjoining fields of bearded wheat. No direct evidence is at hand indicating that the variety of winter wheat grown in this region influences the prevalence of any one physiologic form or combination of physiologic forms.

In table 2 are listed the physiologic forms found in Nebraska during 6 years. (3). In all, 20 physiologic forms have been identified. Of these 20 forms, a number have been found only once during 1 year, while a few have been collected in fairly large amounts during only 1 season. Four forms have been found during 2 seasons, 3 for 3 years, 2 for 4 years, and 2 forms have been found in 5 out of the 6 years studies have been in progress. In other words, there are common and uncommon forms. Physiologic forms 11, 18, 34, and 36 can be classed as common or prevalent forms, while forms 10, 21, and 56 have been present in 3 out of 6 years.

The simultaneous appearance of a physiologic form over a wide area in June indicates that barberries are not the primary source of inoculum. During some years the same form present over a wide area has also been found near infected barberries, but this form must have been present the preceding year, since viable telia must overwinter near-by in order to produce infection of the barberry. The uncommon forms for the most part are apparently forms localized near barberries, since they have not been found in Kansas or southward. The common or prevalent forms present in

TABLE 2.—*Annual occurrence of physiologic forms of Puccinia graminis tritici in Nebraska, 1924–1930*

Physiologic form No.	Number of times collected						
	1924	1925	1926	1928	1929	1930	Total
3	12			17			2
6	1						1
10	4		3[a]	4[a]			3
11	34		13[a]	9	8[a]		4
12	2			5			2
15						2[a]	1
17	3						1
18	13	8[a]	50[a]	15[a]	14[a]		5
21	5[a]	4[a]		3			3
24	2						1
25	1						1
29	2						1
31		5					1
32	3	1					2
34	9		7[a]	5[a]	6[a]	10[a]	5
36	11	20[a]	1			48[a]	4
38				1		10[a]	2
39					35[a]		1
49						2[a]	1
56				6[a]	40[a]	3	3
No. of forms ... 20	14	5	5	9	5	6	
No. of collections	102	38	74	65	103	75	

[a] Forms also found in Kansas and southward.

Data for 1924, 1925, 1926 obtained from Nebr. Agr. Exp. Sta. Res. Bul. 52.

Nebraska in any one year have in most instances been found also in Kansas, Oklahoma, and Texas (2).

Another striking relation is that the common or prevalent forms are rather akin to each other, varying in their reaction on 1 or 2 of the differential hosts. Thus, forms 3, 18, and 36 differ only in their reaction on Kubanka, forms 10 and 39 on Marquis, 21 and 34 on Kanred, and 11 and 15 where the type of infection on some of the differential hosts differs only in some minor degree.

It is of interest at this point to compare the physiologic forms found in Nebraska and southward with those identified by Stakman, Levine, and Wallace (5) and Wallace (6). A direct comparison for the years 1926

and 1928 can be made. In Nebraska forms 10, 11, 18, 34, and 36 were determined from 74 collections (Table 2). Stakman *et al.* from 8 collections identified 3 physiologic forms, namely, 18, 21, and 36. In Texas they found 7 forms present, 11, 17, 18, 19, 21, 36, and 39. From 4 collections sent from Texas, Peltier and Thiel (3) obtained forms 11 and 10, a form closely allied to 39.

Of the 7 forms determined by Stakman *et al.* in Texas, 5 were found in Nebraska by both groups of workers, if we assume that forms 10 and 39 are akin. Although they did not find form 11 in Nebraska, it was determined by us to be one of the most widely distributed forms in the State. The fact that we determined this form from Texas material and again in Nebraska a number of times, using the same methods of identification, obviates the attempt of Stakman, Levine, and Wallace (5) and Wallace (6) to assign form 11, as determined by us, to form 32.

In 1928 9 forms, 3, 10, 11, 12, 18, 21, 34, 38, and 56 were found in Nebraska. Stakman *et al.* identified 7 forms, 17, 21, 32, 36, 38, 39, and 49. Of the 8 forms found by them in Texas, 6 were identified from Nebraska. As in 1926, form 11 was found by Stakman *et al.* in Texas and Kansas but not in Nebraska, although it was identified from 9 collections by the writer.

Thus, while the physiologic forms found in Nebraska and southward were not always the same as identified by others, enough of the same forms were identified by both groups of workers to show that there is a movement of rust spores from the south into Nebraska.

Popp and Craigie (4) report that the first definite spore shower of the season occurred over Manitoba on June 17, 1929, and it was apparently correlated with a sluggish low-pressure area, responsible for a general air movement from the Mississippi Valley into Canada. At this time stem rust was present over all of Kansas and southward and over the greater part of Nebraska. A comparison of the physiologic forms found in Kansas and Nebraska with those later found in Canada should show some similarity if their contentions that spores present over Manitoba originated in this region.

In Kansas and Nebraska the 2 widely distributed forms were 39 and 56, while Newton and her associates (1) found 36 and 49 to be the prevailing forms in Canada. A number of collections of forms 21 and 38 also were made. From 240 collections made in the 3 Prairie Provinces of Alberta, Saskatchewan, and Manitoba they isolated a total of 16 forms.

Form 56, widely distributed in Kansas and Nebraska, was found by them in Canada, while form 39, identified 35 times in Nebraska, was found only once in Canada. On the other hand, form 49, widely distributed in Canada, was not present in Nebraska. Form 36, also found a number of

times in Canada, was not present in Nebraska, unless we consider forms 18 and 36 to be identical, as suggested by Wallace (6). Only 3 forms present in Nebraska and Kanasas in June were later found in Canada, so that the evidence of a spore shower occurring over Manitoba originated from spores in the winter-wheat belt is not conclusive, if it is based on the prevalence of the physiologic forms determined in the 2 regions.

The evidence presented suggests that there is each year a movement of urediniospores from local overwintering centers in the south into Kansas and Nebraska. The amount and distribution of inoculum vary with the prevailing environmental factors each season. This movement can not as yet be traced with certainty, since inoculum also originates from infected barberries yet to be eradicated in these and other States in the winter- and spring-wheat areas of the Mississippi Valley.

SUMMARY

Intensive studies of the primary sources of wheat stem rust in Kansas and Nebraska, based on observations of the time of its appearance, amount, and distribution and on the annual occurrence of physiologic forms of *Puccinia graminis tritici*, were made in 1928, 1929, and 1930.

In the majority of instances infected barberry bushes in northern Kansas were observed spreading *P. graminis secalis* to near-by grains and susceptible grasses. In Nebraska only 1 infected bush was found spreading *P. graminis tritici* prior to the appearance of primary uredinia not directly traceable to barberries.

Primary uredinia not traceable to barberries were found during the first week in June in Kansas and by the second week in southern Nebraska in 1929 and 1930. They appeared somewhat later in 1928.

No estimable loss from wheat stem rust occurred in either State in any of the 3 years.

The physiologic forms identified in Nebraska were found also in Kansas and southward in most instances.

The evidence presented suggests that there is a movement of urediniospores from local overwintering centers in the south, into Kansas and Nebraska each season. The time of the appearance, amount, and regional distribution of this initial inoculum varies with the prevailing environmental conditions.

The exact amount and distribution of wind-blown urediniospores reaching Kansas and Nebraska cannot be definitely determined, since inoculum also originates from infected barberries not yet eradicated in these and neighboring States.

LITERATURE CITED

1. NEWTON, MARGARET, T. JOHNSON, and A. M. BROWN. The seasonal distribution of physiologic forms of Puccinia graminis var. tritici in Canada. Canada Expt. Farms Rpt. **1930** (Rpt. Dom. Bot.): 46–47. 1931.
2. PELTIER, G. L. Some aspects of the spread of stem rust. Centbl. Bakt. [etc.] Abt. II, **78**: 525–535. 1929.
3. ————, and A. F. THIEL. Stem rust in Nebraska. Nebr. Agr. Exp. Sta. Res. Bul. 42. 1927.
4. POPP, WM., and J. H. CRAIGIE. Rust epidemiology. Canada Expt. Farms Rpt. **1929** (Rpt. Dom. Bot.): 37–42. 1931.
5. STAKMAN, E. C., M. N. LEVINE, and J. M. WALLACE. The value of physiologic-form surveys in the study of the epidemiology of stem rust. Phytopath. **19**: 951–959. 1929.
6. WALLACE, J. M. Physiologic specialization as a factor in the epiphytology of Puccinia graminis tritici. Phytopath. 22: 105–142. 1932.

THE TECHNIQUE OF ISOLATION IN MICROBIOLOGY

SYDNEY DICKINSON[1]

(Accepted for publication July 8, 1932)

INTRODUCTION

For a number of years the value of reliable methods of single-cell isolation has been recognized in all branches of microbiology, but only recently has the essential need for accurate methods of single, particular cell isolation[2] become apparent. There is, however, no one method satisfactory for all types of material; consequently, it is proposed to describe a number of methods, some of which are new, which will embrace all types of material, including bacteria and algae. It is proposed neither to describe methods, such as the double-plate method of isolating the sporidia of *Tilletia tritici* (Bjerk.) Wint. (4), which rely on the self ejection of the spore from its hymenium, nor to describe methods dependent on the growth-differentiating properties of chemical substances. Such methods as these are not, in general, sufficiently reliable for single, particular cell isolation. All the methods to be described have a physical background and have been used and tested by the writer.

These methods may be arranged according to the means employed in separating the cells, the one from the other, *viz.*:

I. Cells separated by chance, usually in a volume of liquid, *e.g.*, the dilution and pipette methods, etc.
II. Cells separated by design when lying in one plane, *e.g.*, the dry-needle and Dickinson methods, etc.
III. Cells separated by force, *e.g.*, the microscissors method, etc.

While the methods included in the first 2 sections are applicable to almost all types of material in which cells are not united, the dry-needle method is suitable only for relatively large, thick-wall cells, and the Dickinson method is best suited for rather smaller cells. On the other hand, the methods included in the third section are adapted for dealing with all types of material whose cells are fastened together in filaments, plates, or masses.

It is hoped to describe methods for separating nuclei of different kinds in coenocytic cells at a later date.

I. CELLS SEPARATED BY CHANCE USUALLY IN A VOLUME OF LIQUID

The original means of isolation were the "dilution" or "poured-plate" methods. While there are innumerable variations, these methods depend,

[1] Rockefeller Foundation Fellow at the University of Minnesota, on leave from the University of Wales.

[2] By single particular cell isolation is meant the isolation by choice of single cells distinguished either by reason of their morphology or position of origin, etc.

in principle, on the making of an even suspension of cells of such a concentration that on an average one drop of the suspension will contain only one cell. This suspension is either made in agar before gelation and then poured in a thin layer in a Petri dish, or it is made in a sterile liquid. In the latter case drops of the suspension are put at marked places on the surface of nutrient agar in a Petri dish. Incubation follows, and, as soon as possible after germination, the plates are examined under a microscope. Colonies that have arisen—as far as can be ascertained—from one cell and that are well separated from other colonies are then cut out from the agar and transferred to stock-culture tubes.

In the case of bacteria, owing to the advisability of determining at once, by direct observation, the presence of an isolated cell, films are made either of agar or of gelatin in various ways on cover slips, etc., or, as in the modified Burri's method (22) on celluloid strips with Indian ink. One drop of a suitable dilute suspension is placed on the agar film or mixed with the Indian ink, and a microscopic examination is made at once to determine whether the isolation of a cell has been achieved. Topley, Barnard, and Wilson (28), after covering the isolated bacterium with a mercury droplet, exposed the film to ultra-violet light and so killed the rest of the bacteria.

To help recognition of the microscope field during and after incubation, Ørskov (21) scratched his cover slips with a diamond, and Gardner (13) used ash prepared by burning a loopful of serum. To facilitate the removal of agar blocks containing isolated cells or colonies, Keitt (18) has devised two instruments, while Ezekiel (12) has suggested modifications of his method. One of these tools is designed for the cutting out of cylinders of agar and the other for lifting these cylinders out of the surrounding agar and transferring them to the stock culture tubes. The instrument used by Sass (25) served for both these purposes. La Rue (19) has invented a "cutting out" device which is screwed into the nose-piece of a microscope. This enables the agar cylinders to be quickly and easily delimited. With bacteria, Ørskov (21) used a "harpoon" method for subculturing the growth of single-cell colonies from his agar films, while Gardner (13) improved this method by arranging for the whole process of subculturing to be carried out under microscopic observation. A review of the four most practical and widely used methods in bacteriology has recently been made by Stoughton (27).

The chief objections to the dilution method and its variations are: firstly, that there is no absolute guarantee of the origin of a colony from a single cell, unless the growth has been continuously observed under the microscope; secondly, that single cells only are isolated; and lastly, that a considerable amount of time and labor is consumed.

The first step in the direction of greater reliability was the "Barber pipette" method (2). Originally this was devised for use with bacteria, but it has been applied with success to fungi, algae, etc. Described first in 1904, in principle it consisted in the taking up of a single cell (or bacterium) from a drop of liquid on a cover slip inverted over a modified van Tieghem cell into a micropipette under the microscope. The end of the pipette containing the drop of liquid was broken off and dropped into a flask or tube containing a sterile nutrient medium. The chief difficulty in this method is the cleansing and greasing of the cover glasses, so as to allow small enough water drops to be formed. A recent and full description of this method has been written by Gee and Hunt (14). While it is reliable, though slow and difficult, for bacteria, it can not be used to any great extent where single particular cells are to be isolated. Edgerton (11) has modified the Barber pipette method in using a closed capillary, and so, isolated single ascospores or asci of *Glomerella cingulata*. The spores were sucked into the capillary and ejected by the application of ether and heat, respectively, to the closed end of the pipette. Recently, Craigie (7) has described a method of catching the sporidia of rust fungi, as they are produced, by means of a pipette for this purpose. Ashworth (1), likewise, has used a pipette method for the same type of work.

Schouten (26), somewhat later than Barber, described a method that requires as the vehicle of isolation the film of liquid formed across a loop of very small dimensions. Owing to the difficulties of making such a loop, the method has not been adopted extensively by bacteriologists, but an extension of the method, using larger loops, has been used with success by Dunn, working with *Sclerotinia* spp. (10).

II. CELLS SEPARATED BY DESIGN WHEN LYING IN ONE PLANE

Now it will be appreciated that the isolation of the separate cells by the methods previously mentioned has depended upon use of a liquid in which the cells are separated in a haphazard manner.

When the cells to be isolated are relatively large and dry, *e.g.*, spores, the most satisfactory method is that known as the dry-needle method, described by Hanna (15). This method depends on the fact that a spore will adhere to the point of a dry needle, although (Hanna used a steel needle) the reason for adhesion is not clearly understood. Hanna showed that it was not the ordinary electrical phenomenon of the attraction of oppositely charged particles. It may be that spores, unless specially dried, have a surface film of water (or mucilage) and that the adhesion is due to surface tension. In the writer's experience, absolutely dry spores will not adhere to a dry needle in a dry atmosphere. They will adhere, however, if there is a very small drop of water at the needle tip.

In practice the material—in Hanna's case, basidiospores from a gill of Coprinus—is allowed to fall or is dusted onto a microscope slide. The needle point is brought up to a suitable spore by hand under the microscope. The spore adheres to the needle, and it is then rubbed onto the surface of the agar in a test tube. The lower limits of size of spores for this method are about 20–30 μ. (Fig. 1, A.)

For wet material or for very dry spores, it has been found best to place a small mass of the material on a microscope slide and to add a drop of sterile water. Then, using a low-power microscope and two mounted steel

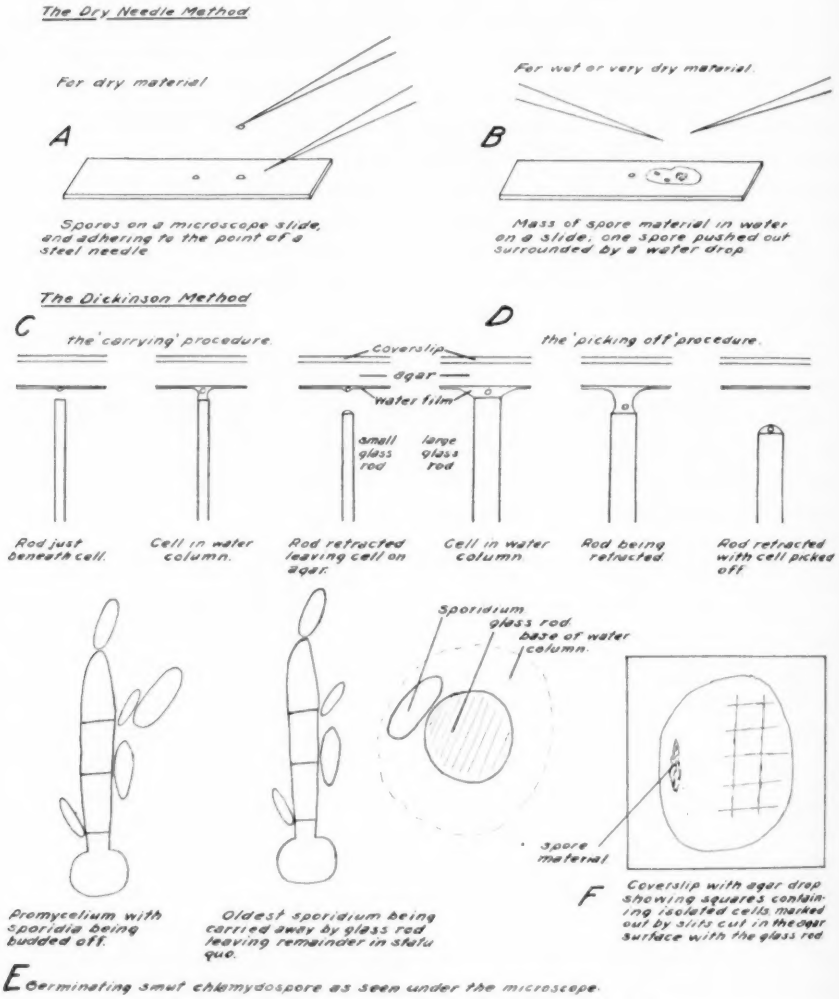
The Dry Needle Method
For dry material.
A
Spores on a microscope slide, and adhering to the point of a steel needle
For wet or very dry material.
B
Mass of spore material in water on a slide, one spore pushed out surrounded by a water drop.
The Dickinson Method
C
the 'carrying' procedure.
D
the 'picking off' procedure.
Coverslip
agar
water film
small glass rod
large glass rod
Rod just beneath cell.
Cell in water column.
Rod retracted leaving cell on agar.
Cell in water column.
Rod being retracted.
Rod retracted with cell picked off.
Promycelium with sporidia being budded off.
Oldest sporidium being carried away by glass rod leaving remainder in statu quo.
Sporidium
glass rod
base of water column.
spore material
F
Coverslip with agar drop showing squares containing isolated cells marked out by slits cut in the agar surface with the glass rod
E Germinating smut chlamydospore as seen under the microscope.

FIG. 1. Dry-needle and Dickinson methods of isolation.

needles, a cell is separated from the mass, and pushed out of the drop of water along the surface of the microscope slide. A small drop of water will be carried with the cell. This drop of water is allowed to evaporate almost entirely, and the cell may then be picked off by a dry needle point. This method can be used only under a low-power microscope and requires a certain delicacy of touch in handling the needles. (Fig. 1, B.)

For small cells, from 30–20 μ down to the limits of microscopic vision, the most satisfactory method appears to be the Dickinson method (8, 9).

In practice, the method consists in "carrying" the cells by means of a fine glass rod from their original position on the surface of an agar drop on a cover slip to the opposite side of the agar drop. The new positions are marked by slits made in the agar surface with the glass rod. (Fig. 1, F.) The pieces of agar containing the isolated cells are cut out and then trans-

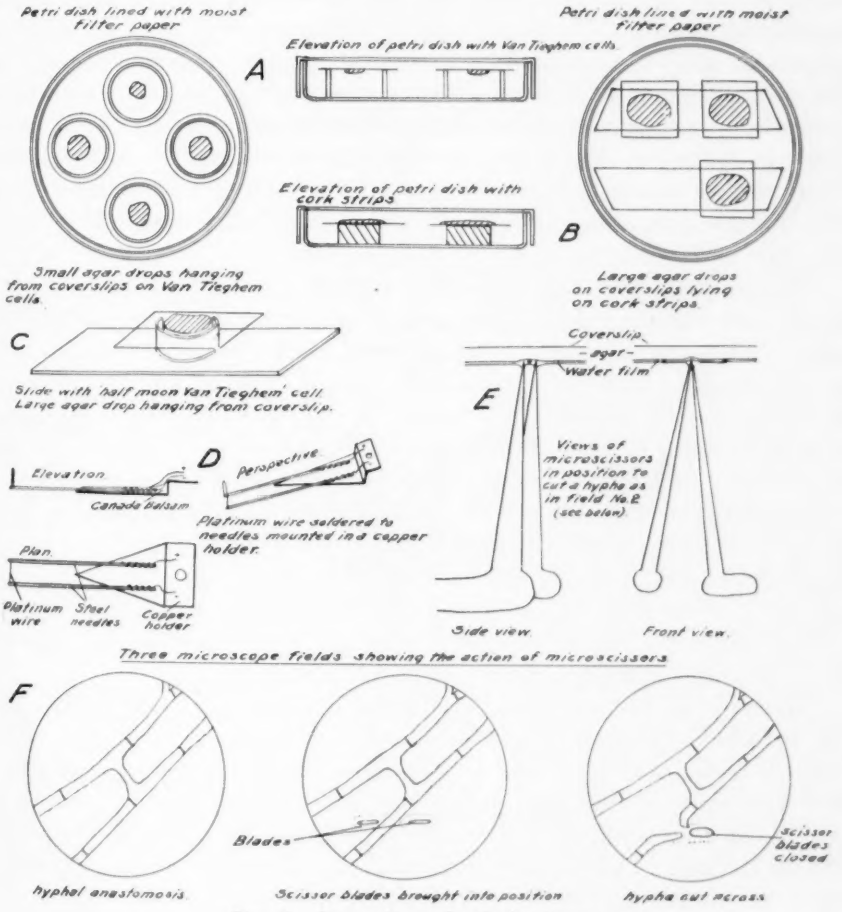
Petri dish lined with moist filter paper
A
Elevation of petri dish with Van Tieghem cells
Elevation of petri dish with cork strips
Petri dish lined with moist filter paper
B
Small agar drops hanging from coverslips on Van Tieghem cells
Large agar drops on coverslips lying on cork strips
C
Slide with half moon Van Tieghem cell. Large agar drop hanging from coverslip.
Coverslip
agar
Water film
E
Views of microscissors in position to cut a hypha as in field No. 2 (see below)
Elevation
Canada balsam
D
Perspective
Platinum wire soldered to needles mounted in a copper holder.
Plan
Platinum wire
Steel needles
Copper holder
Side view
Front view
Three microscope fields showing the action of microscissors
F
Blades
Scissor blades closed
hyphal anastomosis
Scissor blades brought into position
hypha cut across

FIG. 2. Separation of cells by force.

ferred to stock culture tubes with a sterile scalpel. The movement of the fine glass rod is controlled by some mechanical device (see later), while the cover slip is inverted over a crescent-shape van Tieghem cell fastened to a microscope slide (Fig. 2, C), and this is moved by hand or by a mechanical stage.

This method is suitable for the separation and isolation of particular cells in a growing culture because it allows the separation of a particular cell without altering the relative position of any of the neighboring cells, no matter how close they may be to the required cell. (Fig. 1, E.) That this method is speedy is shown by the fact that it is comparatively easy to isolate between 100 and 200 single cells (average length of longer axis 10 μ) in 8 hours.

The principle involved in this method is the utilization of the column of water that can be formed between a glass rod and the film of water on the surface of agar. The reason that a cell can be carried along in such a column of water is that the direction of the applied force, *i.e.*, the edge of the water column, is nearly parallel to the place of motion. When the column of water is very short, the direction of the applied force is at an angle to the place of motion. In such a case the surface tension of the water column surface is not sufficient to overcome the friction between the cell and the agar, and, in consequence, the cell is left behind. (Fig. 1, C, and D.)

Theoretically, a cell can not be picked off the agar by the glass rod unless the diameter of the rod is rather more than 3 times the longer axis of the cell. This has been verified by experiment. What happens when a cell is picked off onto a glass rod is that, with a rod of sufficient diameter, there is room for the cell to sink (by gravity) to the top of the rod. When the column of water is lengthened by the withdrawal of the glass rod, the cell is not drawn back onto the agar surface. In the case of a glass rod of insufficient diameter, while the cell may sink to the top of the glass rod at first, when the column of water is lengthened by the withdrawal of the rod, the cell is drawn back onto the agar surface, as there is not enough room for it on the top of the rod. It should be realized that the glass rod takes off only a water drop of a maximum volume equal to a hemisphere whose diameter is equal to that of the rod itself. (Fig. 1, C and D.)

Hanna (16) has amplified the original description of this method and has advocated, by using a glass rod of sufficient diameter, the picking off of the cells from the original agar drop on the surface of a cover slip and transferring them to a fresh agar drop. For such picking off of the cells, it is best to use a glass rod whose diameter is at least 5 times that of the longer axis of the cell. The writer uses the carrying of the cell in the column of water to another part of the agar for thin-wall cells (Fig. 1, C) and the

picking-off procedure for thick-wall cells. (Fig. 1, D.) The better procedure is, in general, the carrying rather than the picking off of the cells, because there is usually an appreciable number of cases of damage to the cells when they are picked off. It is thought that this is largely due to the evaporation of the surrounding water drop. As regards speed and ease of use, there is very little advantage in either procedure.

In order to be able to pick off cells easily, there must have been very little evaporation from the agar surface. To guard against this, Hanna advocated the use of small drops of agar on the surface of cover slips kept, when not in use, inverted on van Tieghem cells in Petri dishes lined with moist filter paper. (Fig. 2, A.) In the carrying procedure, the writer usually employs larger agar drops (about 2 cm. in diameter) and keeps them under less humid conditions on cork strips in Petri dishes lined with damp filter paper. (Fig. 2, B.)

It is not usually possible to isolate more than about 30 cells on an agar drop when using the carrying procedure. But where a greater number is required, the writer, after transferring the agar pieces containing the already isolated cells to stock-culture tubes, adds fresh agar to the cover slip and proceeds to carry more cells onto this new agar, etc. The only care, besides precautions against contamination, is to see that the surfaces of the old and fresh agar are level. It is as easy to pass from old to fresh agar as it is to pass from one part of an agar surface to another. It is not, however, in general, easy to pass over any place where the agar surface has been cut.

III. CELLS SEPARATED BY FORCE

The isolation of parts of filaments or masses or of any particular cell from a hypha or from cell tissue is complicated, as Harder found (17), by the need for separation as well as for isolation. As regards the separation or cutting out of a cell from a hypha, this is easily done by using a platinum wire (diameter 0.0003 in.) heated by a weak electric current (Fig. 2, D, for method of mounting wire). This cannot, in general, be advised because of the danger of damaging the wanted cell by heat (*cf.* Barnes, 3), but it has been used with success in separating the clamp-connection cells in *Coprinus* spp. Another method of cutting out single cells of hyphae is by means of a small piece of a razor blade, suitably mounted in a micromanipulator or isolator. The film of agar on which the hypha rests must be very thin, as there must be a hard surface against which the razor blade can press. As soon as a thin film of this sort is used, there comes the difficulty of guarding against the drying out of the film, as well as the preserving of the adjacent hyphae in *statu quo*. Lindegren (20) used a tool in the form of a Dutch hoe, made from chromel wire, for the cutting out of single ascospores from the asci of Neurospora. He was able to cut the

ascus wall with this tool because his material was dried and was lying directly on a glass cover slip.

For the isolation of parts of filaments, Brown's "hyphal-tip" method (5) is commonly employed. This, in essentials, is a modification of Edgerton's pipette method. It consists in the cutting out, by means of a fairly fine glass tube, of a cylinder of agar containing one hyphal tip under the microscope and then the blowing out of the agar from the glass tube into a stock-culture tube. This method, provided the hyphae are spaced sufficiently far apart and provided not too small a diameter tube be used, is both quick and easy. It is, however, limited in scope to the tips of growing hyphae. Harder (17) was successful in separating the clamp cells and basal cells in Pholiota. He killed one or the other of the component cells of the developing clamp connection by piercing them with a fine glass rod. He then isolated this cell by dragging away all the other filaments in its neighborhood. This procedure is not generally applicable and requires a high degree of skill.

The method devised by the writer is based on a suggestion made by E. C. Stakman and consists in cutting the hyphae in two with a pair of microscissors attached to a micromanipulator or isolator. The blades of these scissors are made of small pieces of an ordinary razor blade. They are mounted at the ends of circular steel rods. One of the blades moves by a rack-and-pinion movement past the other blade, parallel to the microscope stage. The other blade can be adjusted as to its height above the microscope stage, as well as its angle to the perpendicular. The result is that the blades can be adjusted so that their tips are on the same level and that these tips come in contact only when the movable blade passes the stationary one. (Fig. 2, E.) In addition, the pressure between the tips of the blades, on contact, can be adjusted.

The above-described adjustments are important because there is always a tendency for hyphae to slip between the blades or between one blade and the agar, rather than to be cut in two. Again, the tips of the blades have to be ground to the finest possible point, or about 5–10 μ in diameter. In general, it is possible only to cut hyphae growing on the surface of agar, but they can be cut with accuracy and without disturbing them, at all, provided the tips of the blades be ground to a really fine point. It must be emphasized, however, that the adjustment of the blades relative to one another is most important.

After the microscissors have been used for some time, it becomes necessary to clean the tips of the blades from adherent bits of agar. This is most easily done by regrinding the points. A couple of strokes along the oilstone with each surface is usually sufficient for the purpose.

In practice, the material is placed or is grown on agar drops on coverslips similar to those used in the isolation of small separate cells (Section II). After deciding on an individual hyphal cell, or, for that matter, a cell formed by the anastomosis of 2 adjacent hyphae, it is perfectly easy to separate this wanted cell by cutting the cells on either side of it. (Fig. 2, F.) Having completed the separation of the required cell, it is necessary to isolate it. This can be done with cells up to about 20 μ long with a glass rod, using the Dickinson method of isolation. Care, however, is necessary, because the cell ought not to be bent at all. While no case of mechanical damage has yet been proved to have occurred, the possibility must always be kept in mind. It seems best to use the carrying rather than the picking-off procedure because, in the latter, there is, first, the danger of drying out, and second, the cell walls at each end of the required cell are liable to stick to the glass rod, thus making it difficult to free the cell from the glass rod. While the transport of single hyphal cells is comparatively easy, when they are only about 20 μ in length, it becomes a matter of considerable difficulty in *Coprinus* spp., for example, where the cells are much longer. In such a case a satisfactory method seems to be to grip a piece of dead adjacent cell between the scissor blades and drag the cell along the surface of the agar until it is on a fresh part of it.

Micromanipulators,[3] *etc.* Numerous machines hvae been used or described by various workers (6, 16, 23, 24, etc.) for handling the pipettes, needles, glass rods, microscissors, etc., but it is not proposed to enter into a description of these various machines nor comment on their suitability. It must be pointed out, however, that there is no necessity to use a micromanipulator or isolator, except where material of small size is involved. The essentials required of a machine are to-and-fro and back-and-forward movements, in order to center the particular tool, and a fine vertical movement. The last is the most important of the three.

The writer has used and taught to students single-cell isolation with the following device, which requires no special apparatus at all. The material from which spores are to be isolated is placed on an agar drop on a cover slip, which is then inverted over a hole (Fig. 3, A and B) cut in a microscope slide. This slide is placed on the mechanical stage of the microscope with the hole—about ⅛ inches diameter—directly over the condenser. (Fig. 3, D.) It is possible with delicate handling to manage without a mechanical stage. A cover slip is put on the top of the condenser and bears an L-shape glass rod, the vertical arm of which tapers to a fine point and is fastened to the cover slip by Plasticene. (Fig. 3, C and D.) The tapered tip of the glass rod is centered by hand, or may be centered by the

[3] The machine used by the author is manufactured and marketed by Messrs. C. Baker & Co., 244 High Holborn, London, England.

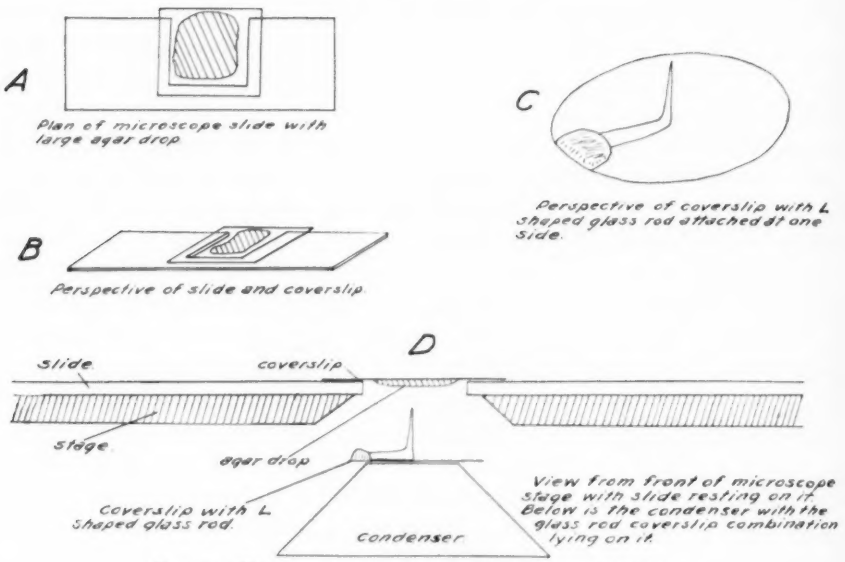
A
Plan of microscope slide with large agar drop
C
Perspective of coverslip with L shaped glass rod attached at one side.
B
Perspective of slide and coverslip
D
Slide
coverslip
stage
agar drop
Coverslip with L shaped glass rod.
condenser
View from front of microscope stage with slide resting on it. Below is the condenser with the glass rod coverslip combination lying on it.

FIG. 3. How to obviate the use of a micromanipulator.

condenser centering screws, if they be present, and is then raised or lowered by means of the condenser rack-and-pinion movement. The actual handling of the spores, etc., is done by using either the dry-needle or the Dickinson method.

SUMMARY

A number of methods for the isolation of single cells have been mentioned and the principles underlying them compared. Those methods that allow the isolation of single particular cells have been described in detail. It is pointed out that for such isolation of medium and large-size separate cells, no special apparatus, other than a microscope, is required.

UNIVERSITY OF WALES,
CARDIFF, WALES.

LITERATURE CITED

1. ASHWORTH, D. *Puccinia malvacearum* in monosporidial culture. Brit. Mycol. Soc. Trans. **16**: 177–202. 1931.
2. BARBER, M. A. A new method of isolating micro-organisms. Jour. Kansas Med. Soc. **4**: 489–494. 1904.
3. BARNES, B. Variations in *Eurotium herbariorum* (Wigg.) Link, induced by the action of high temperatures. Ann. Bot. **42**: 783–812. 1928.
4. BODINE, E. W. Double plate method used for culturing *Tilletia levis*. Science n. s. **74**: 341. 1931.
5. BROWN, W. Two mycological methods. II. A method of isolating single strains of fungi by cutting out a hyphal tip. Ann. Bot. **38**: 402–404. 1924.

6. CHAMBERS, R. New micromanipulator and methods for the isolation of a single bacterium and the manipulation of living cells. Jour. Infect. Diseases **31**: 334–343. 1922.

7. CRAIGIE, J. H. An experimental investigation of sex in the rust fungi. Phytopath. **21**: 1001–1040. 1931.

8. DICKINSON, S. A simple method of isolating and handling individual fungal spores and bacteria. Ann. Bot. **40**: 273–274. 1926.

9. ————. A method of isolating and handling individual spores and bacteria. Proc. Roy. Soc. Med. **19**: 1–4. 1926.

10. DUNN, M. S. The microloop. A rapid method for isolating single spores. Phytopath. **14**: 338–340. 1924.

11. EDGERTON, C. W. A method of picking up single spores. Phytopath. **4**: 115–117. 1914.

12. EZEKIEL, W. N. Modified procedure with the Keitt single-spore method. Phytopath. **20**: 583–586. 1930.

13. GARDNER, A. D. The growth of branching forms of bacilli ("three-point multiplication"). Jour. Path. and Bact. **28**: 189–194. 1925.

14. GEE, A. H., and G. A. HUNT. Single cell technic. Jour. Bact. **16**: 327–347. 1928.

15. HANNA, W. F. The dry-needle method of making monosporous cultures of Hymenomycetes and other fungi. Ann. Bot. **38**: 791–795. 1924.

16. ————. A simple apparatus for isolating single spores. Phytopath. **18**: 1017–1021. 1928.

17. HARDER, R. Über mikrochirurgische Operationen an Hymenomyzeten. Ztschr. Wiss. Mikros. **44**: 173–182. 1927.

18. KEITT, G. W. Simple technique for isolating single-spore strains of certain types of fungi. Phytopath. **5**: 266–269. 1915.

19. LARUE, C. D. Isolating single spores. Bot. Gaz. **70**: 319–320. 1920.

20. LINDEGREN, C. C. The genetics of Neurospora. I. The inheritance of response to heat treatment. Bul. Torrey Bot. Club **59**: 85–102. 1932.

21. ØRSKOV, J. Method for the isolation of bacteria in pure culture from single cells and procedure for the direct tracing of bacterial growth on a solid medium. Jour. Bact. **7**: 537–549. 1922.

22. PAINE, S. G., and J. C. RAMCHANDANI. An improved method of culture from a single bacterial cell. Jour. Bact. **17**: 377–378. 1929.

23. PÉTERFI, T., and L. WÁMOSCHER. Die Isolierung von Bakterien im Dunkelfeld: Ein-Zell-Kulturen und Tierimpfung mit einem einzelnen Bacterium. Ztschr. Hgy. u. Infectionskrank. **106**: 191–201. 1926.

24. ROBERTS, J. W. A method of isolating selected single spores. Phytopath. **13**: 558–560. 1923.

25. SASS, J. E. The cytological basis for homothallism and heterothallism in the Agaricaceae. Amer. Jour. Bot. **16**: 663–701. 1929.

26. SCHOUTEN, S. L. Reinkulturen aus einen unter dem Mikroskop isolierten Zelle. Ztschr. Wiss. Mikros. **22**: 10–45. 1905.

27. STOUGHTON, R. H. The isolation of single bacterial cells. A system of bacteriology in relation to medicine. **9**: 100–109. H. M. Stationary Office, London. 1931.

28. TOPLEY, W. W. C., J. E. BARNARD, and G. S. WILSON. A new method of obtaining cultures from single bacterial cells. Jour. Hyg. **20**: 221–226. 1921.

GIBBERELLA MONILIFORMIS ON CORN

R. K. VOORHEES[1]

(Accepted for publication June 11, 1932)

INTRODUCTION

Perithecia of *Gibberella moniliformis* (Sheld.) Wineland are produced on corn-leaf sheaths under natural conditions in Florida. The imperfect stage of this fungus, commonly referred to as *Fusarium moniliforme* Sheld., is a parasite on corn throughout the world (1, 2, 3, 7). In the southern part of the United States, where the climate is warm and humid during the latter part of the corn-growing season, the disease caused by this fungus is very destructive. In Florida the annual reduction in yield caused by *G. moniliformis* is estimated at 5 per cent of the corn crop.

This report deals with a description of the symptoms of the above-mentioned disease, as it was observed in the favorable environment of Florida, and studies pertaining to the characteristics of the causal organism, its pathogenicity, and the natural occurrence and artificial production of its perfect stage.

SYMPTOMS

In Florida, after corn plants have matured and the leaves begin to dry, the leaf sheaths and culms of many plants become covered with the pink spore masses of the Fusarium stage of the fungus, and the growth of the organism in the stalks sometimes so weakens them at the lower nodes that they are easily broken by the wind. Ear worms, which tunnel through the husks and shanks or enter the ears at exposed tips, open a pathway for the parasite to enter, resulting in moldy kernels adjacent to those damaged by the insects. Sometimes, all of the kernels of a diseased ear are covered with mycelium of the fungus, but usually the infected ears show no external signs of the disease and it is detected only when the kernels are tested in a germinator (Fig. 1, A). The damaged kernels either fail to germinate or produce weak seedlings that become covered with pink spore masses of the fungus.

The disease was observed in seedlings grown in sterilized soil inoculated with the parasite. The tops of infected seedlings turned yellow and wilted and the roots and mesocotyls became brown and shrunken (Fig. 1, B). Infection resulted either in the death or a stunting of the growth of the plant.

[1] Grateful acknowledgment is made to Dr. A. H. Eddins for his criticisms and suggestions.

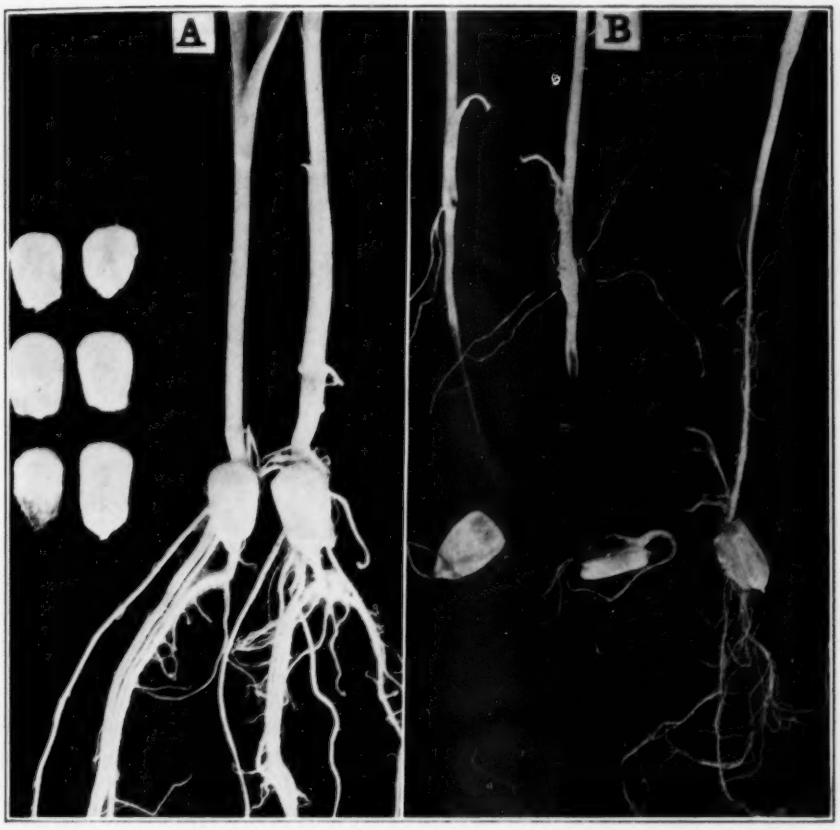

FIG. 1. A. Contrast between kernels naturally infected with *Gibberella moniliformis* (left) and disease-free kernels after 7 days' exposure in a rag-doll germinator. B. Seedlings grown in sterilized soil inoculated with *G. moniliformis* (left), and a seedling grown in sterilized noninoculated soil.

CAUSAL ORGANISM

Morphology

The morphological characteristics of the imperfect and perfect stages of *Gibberella moniliformis* growing on ears and stalks of corn and on potato-dextrose agar in pure culture were studied. With a few exceptions these characteristics were similar to and, in some cases, identical with those described by Sheldon (6) and Wineland (8). The main differences found are pointed out below.

Mycelium. Aerial and submerged mycelium ranged from scanty to medium dense and from dirty white to deep rose pink and pale hortense violet (4). The hyphae were hyaline, septate, and branched. On the host

the mycelium and spores of the fungus appeared as a white to pink felty mass.

Conidia. The simple and branched conidiophores formed at the tips and lateral branches of hyphae bore the microconidia either singly in long chains, or in heads or clusters. Microconidia from corn-leaf sheaths measured 6–15 × 2.5–5 μ, averaging 10.5 × 4.2 μ, and those formed on potato-dextrose agar in culture measured 4.5–12 × 2.5–4.5 μ, the average being 8.9 × 3.4 μ. The spores germinated within 12 to 24 hours in tap water at room temperature, and during germination they swell, usually become 1 to 2-septate, and send out a germ tube from each or only one of the cells.

The macroconidia are borne on aerial mycelium, in pseudopionotes or sporodochia. The spores are somewhat curved, especially near the apex, slightly constricted at the septa, and are 3 to 5-septate, the 3-septate spores predominating. Mature macroconidia from host material measured 26–55 × 3–5 μ, averaging 41.1 × 4.3 μ, and those from potato-dextrose agar measured 24–40 × 3–5 μ, the average being 31.5 × 3.7 μ. Upon germination the spores

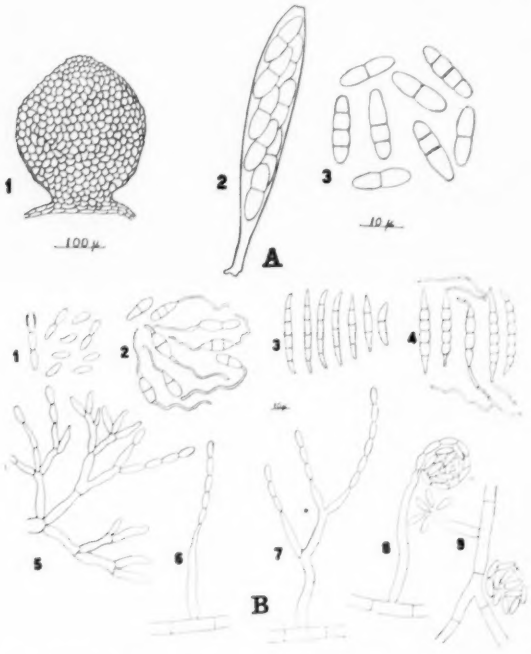

FIG. 2. Camera-lucida drawings of fruiting structures of the perfect and imperfect stages of *Gibberella moniliformis*. A. Typical perithecium, ascus, and ascospores produced on cornstalks. B. Microconidia and macroconidia produced on the host plant and potato-dextrose agar: 1, Typical microconidia; 2, germinating microconidia; 3, typical macroconidia; 4, germinating macroconidia; 5–7, various types of conidiophores; 8–9, clusters of microconidia at tips of conidiophores and along the mycelium.

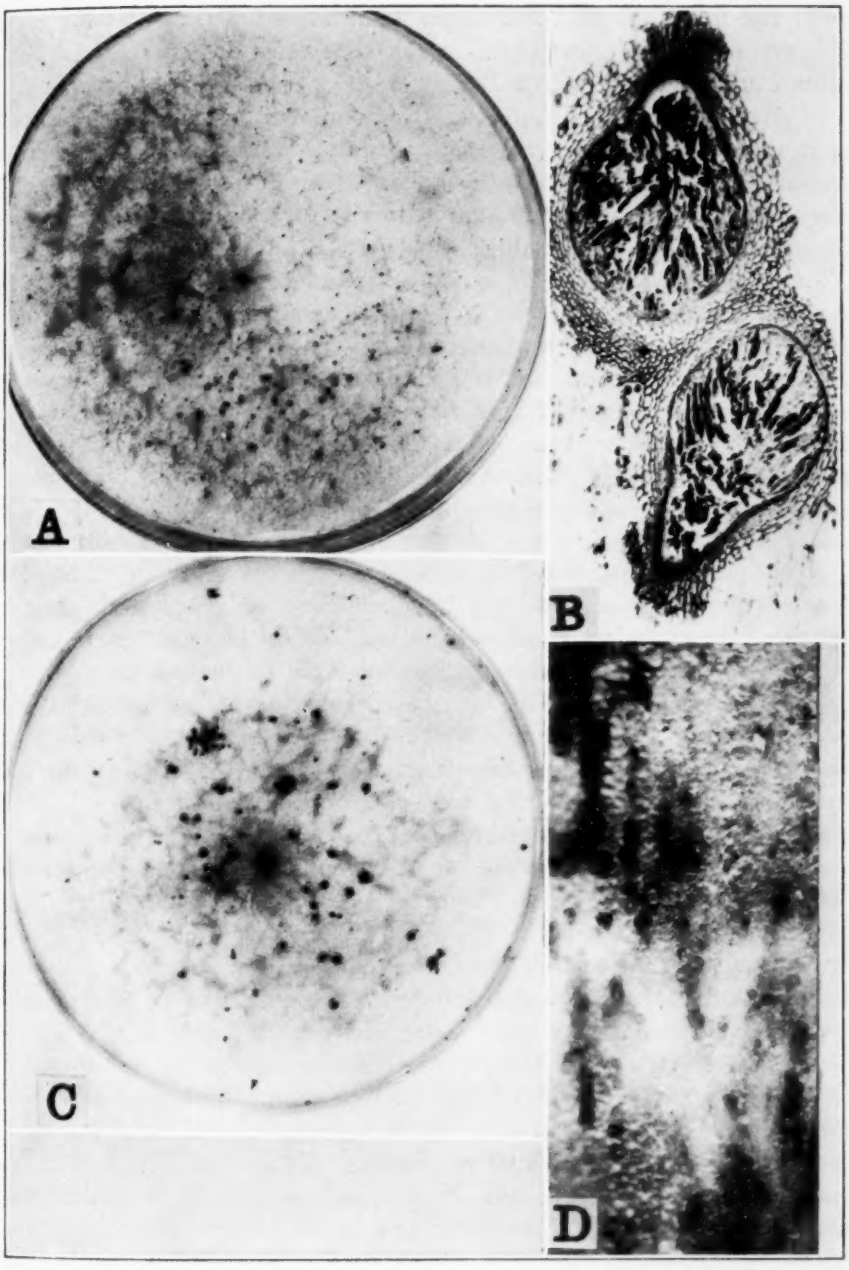

FIG. 3. A–C. Single-ascus cultures of *Gibberella moniliformis* on potato-dextrose agar. Black dots are perithecia. A. Top view. B. Photomicrograph of longitudinal section of perithecia. ×200. C. Bottom view. D. Photomicrograph of perithecia on cornstalk. ×10.

swell and usually become more septate and more constricted at the septa. The germ tubes may form from one or both ends of a spore and become septate and branched (Fig. 2, B).

Perithecia. Perithecia produced in pure culture on potato-dextrose agar were scattered or gregarious, free on the surface of the medium or embedded in the mycelium, and measured 250–300 × 300–350 μ (Fig. 3). Perithecia on cornstalks were scattered or gregarious, erumpent or free on the surface, and measured 210–300 × 275–380 μ (Fig. 3, D).

Ascospores. Each ascus contained 8 ascospores arranged irregularly in 2 rows. The spores were almost straight, rounded at the ends, and constricted at the septa, 1–3 septate, the 1-septate predominating (Fig. 3, B). Ascospores from host material and culture media were practically the same size, as those from corn leaf sheaths measured 12–20 × 4–6 μ, averaging 15.3 × 5 μ, while those from potato-dextrose agar measured 14–20 × 4–5 μ, averaging 17.5 × 4.7 μ (Fig. 2, A).

Taxonomy

The color of the substratum, formation of microconidia in chains, predominance of 3-septate macroconidia, and sizes of the 2 conidial spore forms of the imperfect stage of the causal fungus identified as *Fusarium moniliforme* agree with Sheldon's description (6) of this organism.

The size and shape of the perithecia, asci, and ascospores, absence of paraphyses, and the predominance of the 1-septate ascospores of the fungus also agree with Wineland's (8) descriptions of these characters for *Gibberella moniliformis,* the perfect stage of *Fusarium moniliforme.*

Macroscopically, the perithecia of *Gibberella moniliformis* are very similar to those of *G. saubinetii* (Mont.) Sacc., another species, common on corn and wheat in the North. Perithecia of both species are scattered or gregarious and have a bluish appearance. Microscopically, the 2 species are separable on the basis of certain morphological characters. According to Wineland (8), the straight, usually 1-septate ascospores of *G. moniliformis,* constricted at the septa and rounded at the ends and measuring 15–19 × 3.9–4.8 μ, contrast sharply with the curved, 3-septate ascospores of *G. saubinetii,* scarcely constricted at the septa and pointed at the ends and measuring 18–24 × 4–5 μ, as given by Saccardo (5). The macroconidia of the 2 species are similar in some respects and at times are difficult to differentiate. According to Sheldon (6), the macroconidia of *G. moniliformis* are falcate, acute, usually 3-septate, and 25–40 μ long. Those of *G. saubinetii* are fusiform, curved, acute, apiculate, 5-septate, measuring 24–40 × 5 μ, as given by Saccardo (5). The microconidia of *G. moniliformis* are abundant, while those of *G. saubinetii* are very rare.

Production of the Perithecial Stage

Perithecia of *Gibberella moniliformis* have been found in Florida, occurring naturally on corn leaf sheaths. Wineland (8) produced perithecia of this fungus in pure culture by planting paired microconidial cultures of *Fusarium moniliforme* on media and also by combining 2 single-ascospore cultures.

In the present investigations 6 multisporous cultures originating from microconidia and macroconidia of *Fusarium moniliforme* were grown singly and in paired combinations with each other on potato-dextrose agar in Petri dishes. At the end of 65 days perithecia of *Gibberella moniliformis* were observed in 4 dishes in which paired cultures had been planted and were present only in that area on the agar where the cultures had grown in contact with each other. No perithecia were formed in dishes devoted to single cultures.

In July, 1931, cornstalks were inoculated with 9 multisporous conidial cultures of *Fusarium moniliforme*, singly and in paired combinations. This was done by making an incision about 2 in. long in the 4th or 5th internode of a stalk with a downward stroke of a scalpel. Two corn kernels, each having the same or a different culture of the fungus growing on it, were then inserted into the incision and then wrapped with a strip of nurseryman's tape. At the end of 30 days a single culture and 7 of the combination cultures had formed perithecia of *Gibberella moniliformis* on the inoculated stalks (Fig. 3, D).

In September, 1931, 18 corn leaf sheaths bearing numerous perithecia of *Gibberella moniliformis* were collected and a single ascospore from a single perithecium from each sheath was planted in a Petri dish or a test-tube containing potato-dextrose agar. At the end of 20 days several of these single-ascospore cultures produced perithecia, and within 30 days 15 of the 18 cultures had produced perithecia (Fig. 3). Single ascospores from perithecia produced in these cultures were planted and grown on agar and each produced perithecia abundantly, as well as microconidia and macroconidia.

Thus, it is demonstrated that the mating of the proper conidial cultures results in the production of perithecia, that a single conidial culture may form perithecia, and that the majority of single-ascospore cultures produce fruiting bodies of the perfect stage.

PHYSIOLOGY

Growth on Media. The fungus grew well at 30° C. on potato-dextrose, oatmeal, rice, cornmeal, and Lima-bean agars. The microconidia and macroconidia varied greatly in size on the same media, as well as on different media. Microconidia were produced abundantly on all of the different

media, while macroconidia were abundant only on potato-dextrose and oatmeal agars

Relation of Temperature to Spore Germination. Microconidia were germinated in water in drop cultures exposed to temperatures ranging from 5 to 39° C. in electrically controlled incubators. As noted in table 1, the optimum temperature for germination at the end of 24 hours appears to be at or near 30° C. Only 5 per cent of the microconidia exposed for 7 days at 11° C. germinated, while no germination occurred at 8° C. during the same period, thus showing that the minimum temperature for germination lies between 8 and 11° C. The maximum was not ascertained but would seem to lie between 35 and 39° C.

TABLE 1.—*Percentage of germination of microconidia of Gibberella moniliformis exposed to different temperatures for 24 hours*

Temperatures in degrees Centigrade	6	8	11	13	18	25	30	35	39
Percentage of germination	0	0	0	0	8	25	42	15	0

Relation of Temperature to Mycelial Growth. A series of 90 plates of potato-dextrose agar were inoculated in the center with uniform bits of fungous mycelium, and 10 plates were incubated for 5 days at each of 9 different temperatures ranging from 10 to 39° C. The diameters of the colonies were measured at the end of 1, 3, and 5 days, and the average diameter of the 10 plates at each temperature is presented in table 2. The optimum temperature for growth in this test was close to 30° C., the minimum between 10 and 14° C., and the maximum between 35 and 39° C.

Relation of Hydrogen-ion Concentration to Mycelial Growth. Gibberella moniliformis was grown on a 2 per cent potato-dextrose agar adjusted

TABLE 2.—*Relation of temperature to rate of mycelial growth of Gibberella moniliformis*

Degrees C.	Average diameter of colonies in centimeters		
	One day	Three days	Five days
10	0	0	0
14	0	1.7	2.5
19	1.0	3.1	4.5
24	1.3	4.2	6.4
28	1.6	5.3	7.2
30	1.7	5.6	8.4
33	1.4	3.5	5.3
35	0	1.9	3.0
39	0	0	0

to 9 different hydrogen-ion concentrations. Five test-tubes, each containing 15 cc. of this medium, were used for each concentration, and the concentrations used ranged from pH 2.1 to 8.1. The adjustments were made by adding the required number of drops of 2N and N/2HCl and N/2 NaOH, the determinations being made with a Youden Hydrogen-ion Concentration Apparatus. After the medium was adjusted it was poured into Petri dishes, inoculated in the center with uniform bits of mycelium, and exposed to a constant temperature of 30° C. for 6 days. According to the increase in diameters of the colonies, as given in table 3, the reaction most favorable for growth was pH 8.1, which probably is very near the optimum. Growth was slow at pH 2.6 and was inhibited at pH 2.1.

TABLE 3.—*The relation of hydrogen-ion concentration of potato-dextrose agar to the rate of mycelial growth of Gibberella moniliformis at 30° C.*

pH	Average diameter of colonies in centimeters		
	Two days	Four days	Six days
2.1	0	0	0
2.6	0	0	1.5
3.6	1.3	2.3	3.5
4.2	1.8	3.2	5.5
4.8	2.3	4.3	6.8
6.4	2.5	5.3	7.8
6.7	2.9	5.8	8.6
7.1	3.1	6.1	8.9
8.1	3.2	6.3	9.0

Pathogenicity

Ears of corn in Florida are seldom found free from infection with *Gibberella moniliformis*. It usually occurs in or on the kernels, and, when other organisms are present, *G. moniliformis* is usually present also.

In January and February, 1931, a test was made to determine the effect of the presence of *Gibberella moniliformis* on the viability of kernels of seed ears of 4 varieties selected from the 1930 crop. This test was made by removing 10 kernels from each ear and growing them in a modified rag-doll germinator. The results are given in table 4. The ears classified as having all strong kernels were those whose kernels formed radicles and plumules of normal size. Ears classified as having weak kernels were those whose kernels developed small pumules without radicles, small radicles without plumules, or plumules and radicles much smaller than strongly-germinating kernels. Ears with dead kernels were those whose kernels did not form radicles and plumules. *Gibberella moniliformis* was present on all dead

TABLE 4.—*The effect of Gibberella moniliformis on the viability of corn kernels germinated in a modified rag doll*

Variety	No. ears tested	Percentage of ears showing			
		All strong kernels	One or more weak kernels	One or more dead kernels	One or more weak and dead kernels
Whatley	229	35	33	20	12
Tisdale	73	56	16	16	12
Cuban Yellow Flint	64	54.5	20.6	11.6	13.3
Powell's White Dent	243	60.8	26	8	5.2

kernels, all those that were weak, and on most of those that showed a normal germination. Since the only organism present was *G. moniliformis,* it is reasonable to assume that it was responsible for the death and reduced vigor of the seed. Its presence on the kernels that germinated indicates strongly that there are nonpathogenic strains of the fungus.

Kernels from ears that germinated 100 per cent in the modified rag-doll germinator were surface sterilized in a 1: 1000 bichloride of mercury solution and planted in sterilized soil inoculated with pure cultures of *Gibberella moniliformis* growing on sterilized corn meal. At the end of 8 days after the seedlings emerged from the inoculated soil their tops were yellowed and wilted, and the mesocotyls and roots invaded by the fungus were brown and shrunken. In most cases infection resulted in the death of the plants within 2 weeks after their emergence from the soil, and seedlings that were

TABLE 5.—*Yields of corn from good seed and seed weakened by infection with Gibberella moniliformis in Florida in 1929 and 1930*

Year	Location of experiment	Number of replications	Yield per acre		Difference per acre due to good seed
			Good seed	Seed weakened by *G. moniliformis*	
				Bu.	*Bu.*
1929	Gainesville	10	21.2	16.1	+5.1
	Quincy	8	48.3	44.8	+3.5
1930	Campbellton	10	17.8	17.2	+0.6
	Gainesville	8	11.9	12.1	−0.2
	Quincy	10	54.9	49.4	+5.5

not killed were much retarded in growth. Plants grown in noninoculated soil had healthy roots and a normal top growth.

Experiments were conducted in 1929 and 1930 to determine the effect on the yield of plants grown from seed weakened by infection with *Gibberella moniliformis*. Such seed, when tested in the germinator, showed 1 or more weak kernels but never more than 1 dead one out of each 10 from each ear used. The good seed germinated 100 per cent strong. The yields in 1929 were obtained from 1-row alternating plots 50 ft. in length, replicated. Yields in 1930 were obtained from the center row of alternating 3-row plots, also replicated. The results, as reported in table 5, show that seed weakened by infection from *G. moniliformis* yielded less on the whole than did good seed. The reduction in yield ranged from −0.2 to 5.5 bu. per acre.

SUMMARY

Gibberella moniliformis is one of the most prevalent corn-seed parasites in Florida. The outstanding symptoms of the disease caused by it are discussed.

The morphological characters of the imperfect and perfect stages of the species of Gibberella investigated agree with the original descriptions given for *Fusarium moniliforme* Sheld. and *G. moniliformis* (Sheld.) Wineland.

The perfect stage was found occurring naturally on corn and was produced on media in pure culture by pairing multisporous cultures derived from microconidia and macroconidia and also by inoculating cornstalks with multisporous cultures singly and in combination. Single-ascospore cultures isolated from corn-leaf sheaths produced perithecia abundantly.

The fungus grew well on standard culture media but did best on potato-dextrose agar, in which it produced both microconidia and macroconidia abundantly.

Microconidia of *G. moniliformis* germinated in water at temperatures ranging between 11 and 35° C., the optimum being close to 30° C. In pure culture on potato-dextrose agar the minimum temperature for mycelial growth of the organism is between 10 and 14° C., the maximum lying between 35 and 39° C. and the optimum near 30° C.

Growth did not occur on potato-dextrose agar at pH 2.1, and the fungus grew best at pH 8.1. The optimum, however, may lie above this, as it was not grown on media at higher concentrations.

Modified rag-doll germination tests of seed ears showed that *G. moniliformis* either weakened or killed the kernels or had no effect on them. The roots and mesocotyls of corn seedlings grown in sterilized soil inoculated with *G. moniliformis* were invaded by this organism and the plants either died or were retarded in growth. In plot tests conducted in 1929 and 1930

the yields from seed infected with this organism were reduced from −0.2 to +5.5 bu. per acre.

FLORIDA AGRICULTURAL EXPERIMENT STATION,
GAINESVILLE, FLORIDA.

LITERATURE CITED

1. ABBOTT, E. V. Diseases of economic plants in Peru. Phytopath. **19**: 645–656. 1929.
2. KOEHLER, BENJAMIN, and J. R. HOLBERT. Corn diseases in Illinois. Ill. Agr. Exp. Sta. Bul. 354. 1930.
3. MENDIOLA, VICTORIA B. The Fusarium disease of corn. Philippine Agr. **19**: 79–106. 1930.
4. RIDGWAY, ROBERT. Color standards and color nomenclature. 43 pp., 53 col. pl.; 1,115 named colors. Washington, D. C. 1912.
5. SACCARDO, P. A. Sylloge Fungorum **2**: 552–556. 1883.
6. SHELDON, JOHN L. A corn mold (*Fusarium moniliforme* n. sp.). Nebr. Agr. Exp. Sta. Ann. Rpt. **17** (1903): 23–32. 1904.
7. WENHOLZ, H., and W. H. DARRAGH. The root, stalk, and ear rot diseases of maize. Agr. Gaz. N. S. Wales **38**: 39–49. 1927.
8. WINELAND, GRACE O. An ascigerous stage and synonymy for *Fusarium moniliforme*. Jour. Agr. Res. **28**: 909–922. 1924.

THE PRODUCTION OF A WHITE FERTILE SALTANT OF HELMINTHOSPORIUM SATIVUM BY MEANS OF ULTRA-VIOLET RADIATION[1]

F. J. Greaney and J. E. Machacek

(Accepted for publication July 18, 1932)

Most of the previous investigations on the effects of ultra-violet radiation on fungi have been concerned with the effect on sporulation and with the determination of the fungicidal action of these rays. As far as the writers are aware, there is no published account of saltation having been induced by this means.[2] The purpose of the present paper is to give the results of studies undertaken to determine the effect of ultra-violet light on the rate of growth and on the frequency of saltation in *Helminthosporium sativum* Pammel, King, and Bakke.

The strains of *Helminthosporium sativum* investigated were isolated from diseased parts of cereal plants in 1930 and 1931. Single-spore isolations were made from stock cultures. From these monosporous cultures subcultures were made on plates of Bacto potato-dextrose agar (20 cc. per plate). The subcultures were placed in a dark incubator maintained at from 22°–24° C. and allowed to grow for 3 days before irradiation was begun.

The source of the ultra-violet light used in these studies was a quartz mercury-arc lamp (Alpine Sun Lamp, A.C. equipment, 110 volts, 60 cycle). The exposures were made at a distance of 35 cm. from the arc. The cultures were irradiated for a 4-minute period on 3 successive days. In 2 experiments a number of 3-day-old cultures were irradiated through ordinary Jena Petri-dish lids, while others were irradiated through vitaglass covers. The vitaglass covers were sterilized before substituting them for the regular Petri-dish lids. Control cultures were held for an equal length of time in the diffused light of the laboratory and at the same temperature as the irradiated cultures. Several monosporous strains of *Helminthosporium sativum* were used in each experiment.

It was found that comparatively short exposures to ultra-violet rays markedly affected some strains of *Helminthosporium sativum* with respect to the rate of growth and the frequency of saltation, while other strains were apparently unaffected. Nonirradiated cultures remained constant

[1] Contribution from the Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Canada.

[2] Since this paper was written, Dr. Hugh Dickson has published an account of "The effects of X-rays, ultra-violet light, and heat in producing saltants in *Chaetomium cochliodes* and other fungi." Ann. Bot. **46**: 339–404. 1932.

and maintained their cultural characteristics. The greatest number of saltants was produced in cultures irradiated through vitaglass, while the number was less in those cultures treated through ordinary Jena Petri-dish lids. Similarly, ultra-violet rays had a greater or less effect on growth rate, depending on whether irradiations were made through vitaglass or Jena Petri-dish covers. It is apparent, therefore, that the most effective rays are limited to definite regions of the spectrum. The activating region of the spectrum has not been determined. In general, the effects of ultra-violet light on susceptible strains of *H. sativum* were a marked suppression of growth, an increase in pigment, and an increase in the rate of saltation.

Most of the saltants of *Helminthosporium sativum* induced by ultra-violet radiation were tested to determine, to some degree at least, their constancy. The saltants have remained true to type. Many seemingly sterile strains, as well as actively sporulating strains, were obtained. In every case, the formation of sterile strains was closely correlated with the loss of color. This character correlation was observed by Stevens[3] in *H. sativum*. Studies to determine the degree of stability of these new strains of *H. sativum* are being continued.

One strain of *Helminthosporium sativum*, designated as R. L. No. 9, was very unstable in culture and saltated freely when exposed to ultra-violet rays. The original culture was obtained from a rotted crown of Marquis wheat. A single conidium isolated from it in February, 1932, gave rise to a culture from which a number of subcultures were made on plates of Bacto potato-dextrose agar and irradiated in the usual manner.

Four days after the final irradiation had been made 5 saltants, which differed very markedly from the parental colony, appeared as sectors in 3 subcultures irradiated through vitaglass. Transfers from these sectors and from the parent colony were made to freshly-poured plates. Mycelium from one of these saltants gave rise to a culture producing only hyaline mycelium and almost colorless conidia; whereas transfers from the parent form gave typical dark mycelium and dark-brown conidia (Fig. 1). Slight differences in morphology and physiology, as well as in growth rate, of the saltant and parent strain were also observed. This albinistic form has been subcultured repeatedly (both from mycelium and conidia) and has been irradiated but has remained unchanged. There seems to be no doubt, therefore, that an albinistic strain of *Helminthosporium sativum* has been permanently established by ultra-violet radiation.

Although there was a distinct loss of color in the saltant, no real difference in pathogenicity between the saltant and its parent was noticeable.

[3] Stevens, F. L. The *Helminthosporium* foot-rot of wheat, with observations on the morphology of *Helminthosporium* and on the occurrence of saltation in the genus. Ill. Nat. Hist. Surv. Bul. 14: 77–185. 1922.

This may be due to the very inadequate means so far available for measuring slight differences in pathogenicity in this organism. From figure 2 it is apparent that the parasitic behavior of the saltant C does not markedly differ from that of the parent form A, from which it was derived. It should be stated that their relative pathogenicity has been tested only to the leaves of young barley and wheat plants grown in the greenhouse. Thus far, no further comparison in pathogenicity has been made between the normal form and the saltant. In order to verify the parasitic behavior of the parent and its white saltant, reisolations were made. In each case the respective organism was obtained.

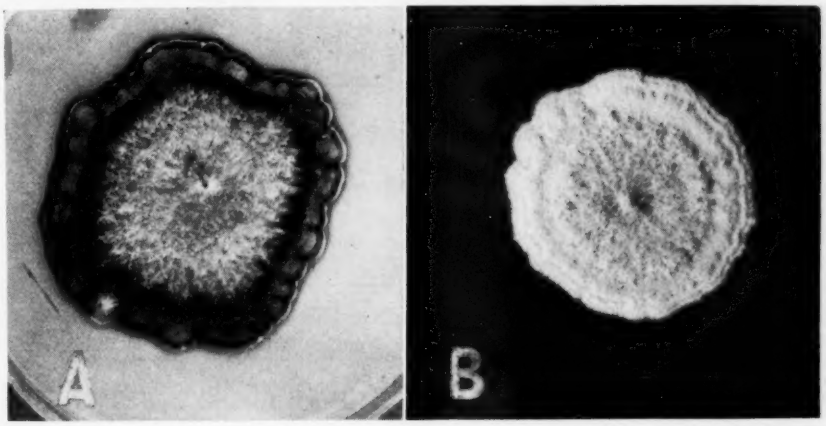

FIG. 1. *Helminthosporium sativum.* Surface views of 10-day-old colonies. A. Dark parent. B. White saltant produced by ultra-violet radiation.

It appears from the studies of many workers that the inherent parasitic capabilities of strains of pathogenic fungi do not change readily. Apparently, most mutations in fungi have been due to the loss of a factor or a group of factors for color. It is of interest that the first conclusive demonstration of mutation in *Puccinia graminis tritici* Eriks. and Henn. was a color mutation. This was reported by Newton and Johnson[4] in 1927. Since that time, however, Stakman, Levine, and Cotter[5] have reported the occurrence in *P. graminis tritici* of a mutation for pathogenicity. It has, therefore, been assumed by the writers that new forms of *Helminthosporium sativum*, differing pathogenically from any forms hitherto known, may occur. The importance of light as a factor in the induction of fungal saltants in nature is relatively unknown, but it appears important enough,

[4] Newton, Margaret, and Thorvaldur Johnson. Color mutations in *Puccinia graminis tritici* (Pers.) Eriks. and Henn. Phytopath. **17**: 711–725. 1927.

[5] Stakman, E. C., M. N. Levine, and R. U. Cotter. Origin of physiologic forms of *Puccinia graminis* through hybridization and mutation. Sci. Agr. **10**: 707–720. 1930.

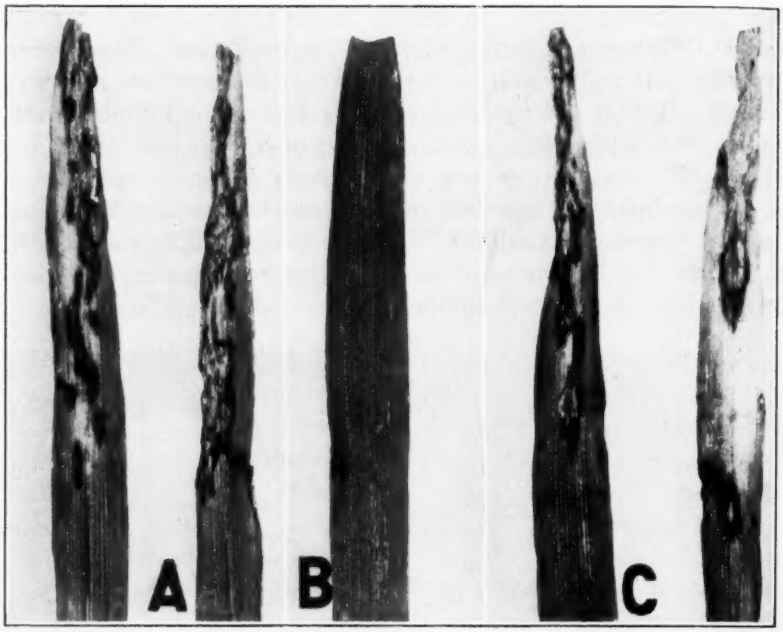

FIG. 2. Leaves of Trebi barley, showing comparative virulence of strains of *Helminthosporium sativum*. A. Dark parent. B. Noninoculated control. C. White saltant.

as shown by the results presented here, to merit further and more detailed investigation.

From a taxonomic view point, the discovery of a strain of *Helminthosporium sativum* with white mycelium and almost colorless conidia is of interest, especially in view of the fact that it is an example of a saltation so great as to remove this form completely from the Helminthosporium group, as described in the taxonomic classification of the *Fungi Imperfecti*. The classification of this new white fungus presents unusual difficulties.

The description of the form-genus Helminthosporium distinctly specifies that the spores or the mycelium are dark. The newly-discovered strain has hyaline mycelium and almost colorless spores, but the spore mass and the mycelium together produce a pale salmon color. As the parentage of this fungus is definitely known, it is convenient to accept it as an albinistic form of *H. sativum*. If this new strain were isolated from diseased plants in nature, it would not in all probability be recognized as a Helminthosporium.

Although white sterile strains of *Helminthosporium sativum* have been frequently obtained in artificial culture by many workers, as far as is known to the writers these strains have never been obtained through direct isola-

tion from diseased plants. If a sterile strain were isolated from a living host, its identity would be difficult to establish by microscopical examination, and, beyond doubt, physiological tests, such as "serum diagnosis," would be required to establish its relationships. It is questionable if even such special tests would be sufficient to identify the fungus.

SUMMARY

A preliminary study has been made of the effect of ultra-violet light on the frequency of saltation in *Helminthosporium sativum*. Cultures of several monosporous strains of this fungus were exposed to the action of ultra-violet light for a 4-minute period on 3 successive days. A few of the strains were apparently unaffected by the rays; the majority produced saltants. The saltants so far have remained constant. In addition to a decided increase in the frequency of saltation, the radiation produced a marked suppression of growth and an increase in pigmentation in those strains affected by the rays.

One strain of *H. sativum* irradiated with ultra-violet light produced a saltant that differed from the parent strain in that the mycelium is hyaline and the conidia almost colorless. In pathogenicity it does not differ appreciably from the parent strain. The difficulty of fixing the taxonomic position of this strain, were it found in nature, is noted.

DOMINION RUST RESEARCH LABORATORY,
WINNIPEG, MANITOBA, CANADA.

OCCURRENCE AND PATHOGENICITY OF NEMATOSPORA SPP. IN FLORIDA

GEORGE F. WEBER

(Accepted for publication June 11, 1932)

Diseased sweet-pepper pods, collected in the vicinity of Miami, Florida, May 20, 1926, contained an organism that, in pure culture, proved to be a species of Nematospora, identical with the illustrations and descriptions of *Nematospora coryli* Peglion described by Peglion (14), as occurring on hazelnuts in Italy. Further work on the organism was not possible at the time and the culture was lost.

On January 10, 1932, H. S. Fawcett found a diseased Satsuma orange on the Florida Agricultural Experiment Station grounds and demonstrated the presence of a species of Nematospora in the affected tissue. He considered it to be identical with the organism previously found by Lee (10) and Fawcett (5) in citrus and other hosts in the Orient and in California. Further collections were made by the writer in a small planting of young bearing satsuma trees, and, by actual counts, 1½ per cent of the fruit on these trees showed such external symptoms as to make it possible to detect the disease. On November 19, 1932, *Nematospora coryli* was found infecting oranges and grapefruit in Pinellas County and, on November 24, on tangerines in Alachua County. The purpose of this paper is to report the occurrence of certain *Nematospora* spp. in the United States and Florida and the results of inoculation experiments.

The infected satsuma fruits always showed more or less drying out in local areas of the rind. The tissue between the oil glands in the affected areas was sunken, causing the oil glands to protrude slightly above the surface. Usually the rind was somewhat wrinkled in the areas invaded by the organism. Not all such fruits, showing the above symptoms, however, were infected. Upon removing the rind, one or more locules showed the infection. In recently infected fruits there were plainly visible whitish spots, circular or irregular in shape and of various sizes located in the locule covering. In older infections the juice sacs within the locule were affected. They showed loss of moisture and often a collapsed condition. In the latter instances one end of the juice sac adhered to the locule wall. Often, in old infections, a discoloration was evident on the locule covering and in the ends of the juice sacs in contact with it. At first this coloration is a lighter color than the noninvaded tissue but later it becomes darker and, when most of the natural moisture has disappeared, the tissue is a bright reddish brown, as observed on other citrus by Fawcett (6). *Nematospora coryli* has been isolated from all stages just described.

While these investigations were in progress a large number of isolations were made from naturally infected satsumas. In a single instance, on February 8, 1932, *Nematospora gossypii* Ashby and Nowell was obtained from a satsuma that had all the characteristics of the disease caused by *N. coryli*. This organism infected the fruit to the exclusion of *N. coryli*. This is, so far as known, the first time *N. gossypii* has been reported outside of the geographical limits of the Lesser Antilles and Trinidad (2), Nigeria (9), and Burma (15). This is also the first report of this organism on citrus fruit of any kind, so far as the writer can determine, although it has been reported previously on cotton, *Datura* sp., and milkweed. Although the diseases caused by *N. gossypii* and *N. coryli* are indistinguishable, the organisms, in culture, are very distinct, as pointed out by Ashby and Nowell (2), Guilliermond (8), and Fawcett and Lee (7).

A single ripe tomato fruit was collected on February 7, 1932, near Homestead, Florida, infected with *Nematospora coryli*. A second shipment of tomatoes received from that section of Florida on February 17 showed 3.6 per cent infected fruit. Another shipment, about March 1, showed 1.3 per cent. The infected fruits in the second and third shipments were in different stages of ripeness, namely, green, pink, and red, indicating that infection took place while they were green. This fungus has been previously reported from tomatoes grown in southern California, Cuba, and Mexico, by Schneider (16, 17), but was named *Nematospora lycopersici*, which is now considered a synonym of *N. coryli* (2). Excepting Schneider's report of its occurrence in southern California, this is the only report of this disease on tomatoes from the United States, although it has been reported on lima beans in Virginia by Wingard (19, 20, 21) and in Illinois by Anderson (1) as *Nematospora phaseoli*, a synonym.

The infections on tomatoes were always associated with a disease previously described (18) as "cloudy spot," which is now considered to be caused by sucking insects of the pumpkin and leaf-footed plant-bug group. Other investigators, Davy (4) and Nowell (12, 13) have intimated and Fawcett (6) has shown that sucking bugs undoubtedly have been the carriers of the *Nematospora* spp. However, in no instance have they isolated the organism from the mouth parts of certain of these insects, even when captured in the act of feeding. The writer made dilution plates of the mouth parts of a dozen individual bugs and obtained *Penicillium* spp., *Aspergillus* sp., *Colletotrichum* sp., *Fusarium* sp., and other fungi, bacteria, and yeasts, but no *Nematospora* sp.

The cloudy spots appear as lighter colored areas anywhere on the surface of tomato fruits. Examination of the tissue shows that it is somewhat pithy or as though the moisture had been withdrawn and the spaces filled with air. When these spots are infected (Fig. 1), the host tissue involved

FIG. 1. Tomatoes naturally infected with *Nematospora coryli* and showing diseased areas.

does not ripen so rapidly as the noninvaded tissue, leaving green islands in a pink or red fruit. These green spots gradually darken as the infection spreads, become sunken, and slowly soften. They are finally invaded by secondary soft-rotting organisms.

Both *Nematospora coryli* and *N. gossypii* grow well on potato-dextrose agar and have been increased on that medium for use in inoculation experiments. Water suspensions were made from cultures of the organisms and were used for inoculating satsuma, tangerine, and tomato fruits in the laboratory. The cultures of *N. coryli* were isolated from satsumas and tomatoes and *N. gossypii* from satsumas. The suspensions contained vegetative cells, mycelium, and ascospores of each of the organisms.

The fruits to be inoculated were disinfected with corrosive sublimate (1: 1000) for 20 minutes; they were then thoroughly washed and placed in moist chambers. Before the inoculations were made the surface of the fruits was swabbed with 95 per cent alcohol and allowed to dry. A large loop transfer needle was then used to place several drops of the inoculum on the sterilized surface, after which a puncture was made with a small sterile needle through the drop of inoculum. The inoculum was usually sucked into the citrus fruits when the needle was removed, while the tomato fruits often exuded juices in droplet form that mingled with the inoculum until the moisture evaporated. Each fruit was inoculated in 10 places and placed in a moist chamber immediately. After 24 hours the tops of the moist chambers were removed and the fruits were left in the open dishes on the laboratory shelf. The checks were similarly treated, except that sterile distilled water was used in place of the inoculum. After 2 weeks the fruits were examined and the data obtained are shown in table 1.

TABLE 1.—*Results following inoculations of satsumas, tangerines, and tomatoes with Nematospora coryli and N. gossypii*

Kind and source of inoculum	Fruits	Inoculated	Diseased
Nematospora coryli (satsuma)	tomato	20	20
	satsuma	12	12
	tangerine	12	10
Nematospora coryli (tomato)	tomato	20	20
	satsuma	12	12
	tangerine	12	12
Nematospora gossypii (satsuma)	tomato	20	16
	satsuma	12	9
	tangerine	12	7
Sterile distilled water	tomato	3	0
	satsuma	3	0
	tangerine	3	0

The area immediately around the points of inoculation dried rapidly and became discolored and shrunken. The culture of *Nematospora coryli* from tomatoes appeared to cause infection of satsumas and tangerines more rapidly than the strain isolated from satsumas. On tomatoes, however, there was very little difference in the effect of the two strains. Both of them produced colonies within the seed cavities and caused a discoloration of the seeds. The multiplication of the organisms was much more rapid in tomatoes than in satsumas or tangerines.

The development of *Nematospora gossypii* in tomatoes was somewhat different from that of *N. coryli* in that it lacked free floating vegetative cells and showed extensive mycelial growth. In this case the mycelium invaded more extensive areas of the inoculated tomato, even growing in among the hairs of the seeds. Ascospores of both species were produced in abundance in infected fruits. Successful inoculations with *N. gossypii* were not so certain as with *N. coryli*. This was also the experience of Marsh (11) in inoculating cotton bolls, although Dastur and Singh (3) obtained a high percentage of infection of cotton bolls with *N. nagpuri* Dast. The organism was recovered in each case of the successful inoculations.

SUMMARY

Nematospora coryli was first found in Florida infecting sweet-pepper pods. It was recently found in this State on satsuma oranges by H. S. Fawcett and on tomatoes, grapefruit and oranges by the writer.

Nematospora gossypii was found on the satsuma orange in Florida for the first time and is the first report of *N. gossypii* in the United States on any host.

This constitutes the first report of both of these organisms on the satsuma orange and of *N. coryli* on peppers in the United States.

Inoculations show both species to be pathogenic on satsuma, tangerine, and tomato.

The isolations of the organisms from tomato and one of those from the satsuma orange are indistinguishable and undoubtedly are *N. coryli*.

FLORIDA AGRICULTURAL EXPERIMENT STATION,
GAINESVILLE, FLORIDA.

LITERATURE CITED

1. ANDERSON, H. W. Notes on the Nematospora disease of lima beans. (Abst.) Phytopath. **14**: 31. 1924.
2. ASHBY, S. F., and W. NOWELL. The fungi of stigmatomycosis. Ann. Bot. **40**: 69–83. 1926.
3. DASTUR, J. F., and J. SINGH. A new Nematospora on cotton bolls in the Central Provinces (India). Ann. Mycol. **28**: 291–296. 1930.
4. DAVY, E. W. European agriculture. Dept. Agr. Nyasaland Protectorate. Ann. Rpt. **1924**: 5–10. 1925.
5. FAWCETT, H. S. Nematospora on pomegranates, citrus, and cotton in California. Phytopath. **19**: 479–482. 1929.
6. ————. Occurrence and insect transmission of Nematospora in California. (Abst.) Phytopath. **19**: 1138. 1929.
7. ————, and H. A. LEE. Inspissosis or dry rot of fruit. Pp. 415–420. *In* Citrus diseases and their control. 582 pp. McGraw-Hill Book Company, New York. 1926.
8. GUILLIERMOND, A. Sur la cytologie des Nematospora. Compt. Rend. Acad. Sci. Paris **185**: 1510–1512. 1927.
9. LAYCOCK, T. Preliminary investigations of the parasitism of certain fungi causing boll rots of cotton. Ann. Bul. Dept. Agr. Nigeria. **4**: 32–49. 1925.
10. LEE, H. A. Dry rot of citrus fruits caused by a Nematospora species. Philipp. Jour. Sci. **24**: 719–733. 1924.
11. MARSH, R. W. Inoculation experiments with *Nematospora gossypii*, Ashby & Nowell. Ann. Bot. **40**: 883–889. 1926.
12. NOWELL, W. Infection of orange fruit through bug punctures. Agr. News **17**: 142. 1918.
13. ————. Diseases of crop-plants in the Lesser Antilles. 383 pp. Diseases caused by fungi. Pp. 28–34. West India Committee, London. [1923.]
14. PEGLION, VITTORIO. Ueber die *Nematospora coryli* Pegl. Centralbl. Bakt. II Abt. **7**: 754–761. 1901.
15. RHIND, D. Preliminary notes on an internal boll disease of cotton in Burma. Agr. Jour. India. **22**: 34–38. 1927.
16. SCHNEIDER, ALBERT. A parasitic saccharomycete of the tomato. Phytopath. **6**: 395–399. 1916.
17. ————. Further note on a parasitic saccharomycete of the tomato. Phytopath. **7**: 52–53. 1917.
18. WEBER, GEORGE F., and G. B. RAMSEY. Tomato diseases in Florida. Fla. Agr. Exp. Sta. Bul. 185. 1926. Cloudy spot. pp. 126–127.
19. WINGARD, S. A. A yeast parasitic on Lima beans. (Abst.) Phytopath. **12**: 47. 1922.
20. ————. Yeast-spot of Lima beans. Phytopath. **12**: 525–532. 1922.
21. ————. Studies on the pathogenicity, morphology, and cytology of *Nematospora Phaseoli*. Bul. Torrey Bot. Club **52**: 249–290. 1925.

APPLICATION OF THE TERMS "ANTHRACNOSE" AND "SCAB" TO PLANT DISEASES CAUSED BY SPHACELOMA AND GLOEOSPORIUM

ANNA E. JENKINS[1]

(Accepted for publication July 14, 1932)

INTRODUCTION

This paper presents the history of the term anthracnose and makes certain suggestions for the use of this and the term scab, particularly as applied to diseases caused by Sphaceloma (including Elsinoë) and Gloeosporium (including Colletotrichum). The treatment is mainly from the standpoint of phytopathologists.

HISTORY OF THE TERM ANTHRACNOSE

Originated to Designate a Sphaceloma Disease of Grapes. To avoid confusion in the term *charbon* as then used in France to designate both a smut of cereals (Gramineae) and the disease of grapes (Vitis) now known to be caused by *Sphaceloma ampelinum* de Bary, Fabre and Dunal (12), in 1853, substituted for the latter disease a new name of the same meaning: "anthracnose (ἄνθραξ, *charbon, et* νόσος, *maladie*)." This is the generally accepted name for the grape disease, although, as listed by Weinmann (38), many other terms besides those named have been applied to it in France and elsewhere. The term anthracnose was thus first used in a symptomatic sense to signify only this one disease, which is hyperplastic in type. *Sphaceloma ampelinum*, described (3) in 1874, was until recently the only species in the form genus Sphaceloma. Burrill (7), Scribner (27), and others considered *Gloeosporium ampelophagum* Sacc. (24) to be the same organism, and this identity was recognized by Saccardo (25, p. 267) in 1906. The classification of the fungus in the genus Gloeosporium led to its being treated as of this genus in many European and American publications and herbaria.[2]

Application to Related Diseases. In accordance with Burrill's (7) discovery of the probable congeneric relationship of the pathogen of what was then known as "blackberry and raspberry cane rust" with *Sphaceloma*

[1] Much of the subject matter presented in this paper is part of a thesis presented to the Graduate School of Cornell University, in June, 1927, in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Thanks are due H. M. Fitzpatrick, C. M. Massey, and A. J. Eames, under whose direction the work was done. Acknowledgment is also made to M. F. Barrus, H. S. Fawcett, H. R. Fulton, W. A. McCubbin, C. L. Shear, H. H. Whetzel, and R. P. White for a critical reading of this paper, especially the parts dealing with suggestions for the application of the terms anthracnose and scab to unnamed diseases.

[2] Observations with respect to European herbaria in part by H. S. Fawcett.

ampelinum, Scribner (29) suggested the name "anthracnose of raspberry and blackberry" for this bramble disease. He (29) explained that the new name was "proposed . . . in uniformity with anthracnose of the vine caused by another species of the same genus." Identifying (29) the bramble-disease pathogen as *Gloeosporium venetum* Speg., he continued to treat the fungus as of the genus Gloeosporium (of which the conidia are generally larger than those of the genus Sphaceloma), although he (27, 28) referred to the pathogen of grape anthracnose as of the genus Sphaceloma. His (30, Figs. 1511 and 1578) illustrations showing the "striking similarity" of these fungi are reproduced in figure 1. Until Burkholder's (4, 5)

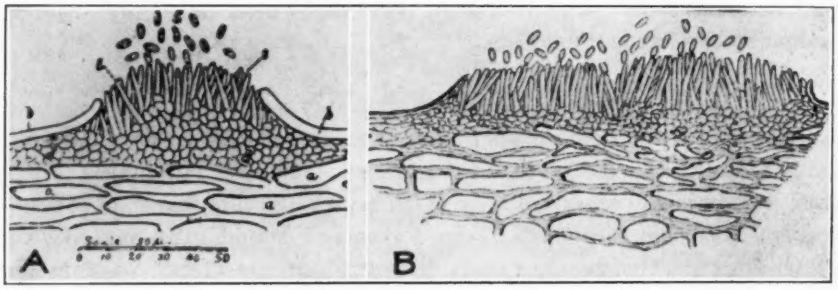

FIG. 1. Conidial fructifications of (A) *Sphaceloma ampelinum* and (B) *Gloeosporium venetum* as illustrated by Scribner (30). These show, as Scribner stated, "the striking similarity of the two fungi."

discovery of the perfect stage of this fungus in 1914, when it was classified as *Plectodiscella veneta* (Speg.) Burk., the bramble fungus was called *G. venetum* and its relationship to *S. ampelinum* rarely mentioned. The two organisms have recently been classified in the genus Elsinoë, the grape fungus as *E. ampelina* (de Bary) Shear (31), the bramble as *E. veneta* (Speg.) Jenkins (16).

The term anthracnose has been applied to four other diseases caused by Elsinoë or Sphaceloma, those affecting rose (Rosa) (17), apple (Malus) and pear (Pyrus), snowberry (*Symphoricarpos albus* Blake var. *laevigatus* (Fernald) Blake), and Labrador tea (Ledum). Cobb (8, opposite page 646) designated the rose disease anthracnose presumably because he thought at the time that its pathogen was a Gloeosporium. As affecting pear, the apple and pear disease was so named by Peglion (20) evidently because of the resemblance of the leaf symptoms to those of grape anthracnose. The early suggestion (36, pp. 393–394) that the snowberry-disease pathogen might be a Gloeosporium doubtless led to the disease being called an anthracnose when it was first reported (36). Barrus and Horsfall (2) later used the same name for the disease, with the explanation that this was

done because of the resemblance of the pathogen to *Elsinoë veneta*, then known as *Plectodiscella veneta*. Zeller and Deremiah (41) did not explain why they applied the term to the Labrador tea disease, but, judging from their paper, this was done because the pathogen is of the same genus (Elsinoë) as that of grape anthracnose.

Application of the Term Anthracnose to Diseases Caused by Gloeosporium and Colletotrichum. In the report containing Scribner's (29) account of anthracnose of raspberry and blackberry, he also discussed anthracnose of the bean (Phaseolus), identifying its pathogen as *Gloeosporium lindemuthianum* Sacc. and Magn. The organism was later transferred to the genus Colletotrichum, as *C. lindemuthianum* (Sacc. and Magn.) Br. and Cav.[3] on the basis of Scribner's (29) observation that it produced setae. This bean disease is often given as an example of a typical anthracnose as well as of a strictly necrotic disease. Scribner (29) apparently applied the term anthracnose to this disease in conformity with the term as used for bramble anthracnose, of which the pathogen was also classified in the genus Gloeosporium. His application of the term to these two diseases constitutes early, if not the first, use of the word for other than the grape disease for which it was originated.

With apparently little thought of its origin, the word has since been in general use for diseases caused by species of Gloeosporium and Colletotrichum and a few genera other than Sphaceloma and Elsinoë. It has also been used to refer to the pathogens of diseases that have been termed anthracnoses. Examples of the use of the term as discussed in this and the preceding paragraph are given below.

According to Stoneman (37), the term anthracnose was "first applied to the 'bird's-eye' fungus of the grape (*Sphaceloma ampelinum* De Bary . . ." and "has since been applied to diseases having similar external appearance and agreeing in general microbotanical characters." Edgerton (11) said that "some [here possibly referring to Shear and Wood (32)] would limit the use of the word anthracnose to those fungi having an ascigeral stage as in the genus Glomerella; but I prefer to use it as commonly used, for all fungi having a Gloeosporium-like conidial stage." He asserted (11, p. 394) that the "majority of the anthracnoses that have been described belong to the genus Glomerella." Shear and Wood (33) said that "in current usage the term 'anthracnose' is applied to diseases caused by fungi belonging to a few other genera besides Glomerella, but that it would be better if the name . . . were restricted to the diseases caused by Glomerella." This genus, they stated (33), is "usually referred to in its conidial stage as Gloeosporium and Colletotrichum."

[3] Briosi, G., and F. Cavara. I fungi delle piante coltivate ed utili, Fasc. 2, No. 50, 1889.

The use of the term anthracnose in naming diseases caused by Gloeosporium and Colletotrichum is further indicated by Rorer's (23) statement that "like other anthracnoses," anthracnose of the mango (Mangifera) "is caused by a fungus belonging to the genus Gloeosporium or Colletotrichum." It is shown also by the fact that the pathogens of most of the 48 anthracnose diseases referred to by Stevens and Hall (34) belong to these two genera and also by Wolf's (40) naming of the vetch (Vicia) disease caused by *Protocoronospora nigricans* (Atk. and Edg.) emend. Wolf "false anthracnose" to distinguish it from what he virtually called the "true anthracnoses" (diseases caused by Gloeosporium and Colletotrichum).

In the 1911 edition of the Century Dictionary[4] the term is defined as referring only to grape anthracnose, "A disease of grape-vines which affects the leaves, young stems, and green berries and is caused by the fungus, *Sphaceloma ampelinum*." In the 1930 edition of the New Century Dictionary,[5] however, the definition is much more general: "A destructive disease of grape-vines, in which brown spots appear on the shoots, leaves, and berries, due to the fungus, *Sphaceloma ampelinum;* also, any of the various other plant-diseases due to fungi." The 1931 edition of Webster's Dictionary[6] defines the term as "A common plant disease caused by several species of Melanconiaceous fungi."

APPLICATION OF THE TERM SCAB TO THESE DISEASES

Although not in common use for them, the term scab, as well as that of anthracnose, has been applied to the diseases of grape (19) and brambles (6) caused by Elsinoë and to the bean disease caused by *Colletotrichum lindemuthianum* (1).

The term is commonly used for the disease of citrus (Citrus) and avocado (*Persea americana* Mill.) (13), caused by Sphaceloma, and those of Lima bean (*Phaseolus lunatus macrocarpus* Benth.) and sword bean (*Canavalia gladiata* (Jacq.) DC.) caused by species of Elsinoë. Scribner (26) named the citrus disease scab obviously because of its hyperplastic scab-like lesions. Stevens (35) called the avocado disease scab on account of its "many points in common" with citrus scab. Following the precedent of Scribner (26), the writer (14, 15) applied the term to the two legume diseases, that affecting Lima bean having already been called "verruga" by Cuban growers of the crop.

[4] Century Dictionary. An Encyclopedic Lexicon of the English Language, prepared under the superintendence of William Dwight Whitney . . . rev. and enl. under the superintendence of Benjamin E. Smith. 1911.

[5] New Century Dictionary of the English Language. Edited by H. G. Emery and K. G. Brewster . . . vol. 1, 1929.

[6] Webster's New International Dictionary of the English Language based on the International Dictionary of 1890 and 1900. Springfield, Mass., U. S. A., 1931.

SUGGESTIONS FOR THE FUTURE USE OF THE TWO TERMS

The general statement may be made that plant diseases are classified either on the basis of the taxonomic position of their pathogens or on their predominating disease processes. Whetzel (39), as well as others, maintains that the latter basis is the "only logical" one. According to him, the etiological basis is objectionable because there is no "consistent correlation between kinds of pathogenes involved and the character of the disease they produce."

As already explained, in naming plant diseases, the term anthracnose is now used chiefly in an etiological sense, although it was originally used only in a symptomatic sense. In the future, where this term and that of scab are applied by pathologists to as yet unnamed diseases of the groups discussed herein, it is suggested that they be used in a symptomatic sense, anthracnose for necrotic and hypoplastic diseases having somewhat restricted lesions as in bean anthracnose and scab for diseases having hyperplastic scab-like lesions as in citrus scab. The diseases of Antidesma and Tinospora (22, t. 1) caused by Elsinoë, for example, which are hyperplastic in type, could thus appropriately be called scab, and the Sphaceloma disease of poplar (Populus) (18), apparently non-hyperplastic, anthracnose.

Of course, neither term should be employed where already in common use for another disease of the same plant, or else the terms should be so qualified that confusion will not result. For other reasons, a different terminology may sometimes be desirable, as in cases where some other particularly descriptive name has been applied to a disease.

The recent application (9, 10) of the name anthracnose to a Gloeosporium disease of snowberry has resulted in a duplication of disease names of this suscept. The term "ripe rot" (21) serves to distinguish the Gloeosporium disease of grapes from that caused by Sphaceloma, and it is suggested that this name may appropriately be used for the snowberry disease caused by Gloeosporium, which also becomes evident upon the ripening of the fruits (10).

By common consent the name of a plant disease should not be used by itself to refer also to its pathogen or, as expressed by Whetzel (39), "disease and pathogene concepts" should not be synonymized. Therefore, it is desirable that the use of the term anthracnose in reference to pathogens of diseases so named be discontinued.

SUMMARY

The term anthracnose was originated by Fabre and Dunal for a grape disease, in order to avoid confusion in the term *charbon* as then employed in France to designate both a cereal smut and the grape disease. The new

name for the grape disease was used only in a symptomatic sense. The pathogen of this disease was later described as *Sphaceloma ampelinum* and is now classified in its perfect stage as *Elsinoë ampelina*. Scribner extended the term anthracnose to denote the related bramble disease the causative organism of which was commonly known in its imperfect stage as *Gloeosporium venetum* and recently classified in its perfect stage as *E. veneta*. He also applied the term to the unrelated legume disease caused by *Colletotrichum lindemuthianum*. As in these two instances, the term has since been applied chiefly in an etiologic sense to designate diseases caused by Elsinoë, Sphaceloma, Gloeosporium, and Colletotrichum and those caused by fungi of a few other genera. Some of these diseases, both hyperplastic and nonhyperplastic, have also been called scab.

It is suggested that, in the future, where this term and that of scab, may be used in naming diseases caused by fungi of the genera named, they be applied in a symptomatic sense, anthracnose for necrotic and hypoplastic diseases with somewhat restricted lesions, as in bean anthracnose, and scab for hyperplastic diseases with scab-like lesions, as in citrus scab.

LITERATURE CITED

1. BARRUS, M. F. Bean anthracnose. New York (Cornell) Agr. Expt. Sta. Memoir 42: 1921.
2. ————, and J. G. HORSFALL. Preliminary note on snowberry anthracnose. Phytopath. **18**: 797–802. 1928.
3. BARY, A. DE. Ueber den sogenannten Brenner (Pech) der Reben. Ann. Oenol. **4**: 165–167. 1874.
4. BURKHOLDER, W. H. The perfect stage of the fungus of raspberry anthracnose. (Abst.) Phytopath. **4**: 407. 1914.
5. ————. The perfect stage of *Glocosporium venetum*. Phytopath. **7**: 83–91. 1917.
6. ————. The anthracnose disease of the raspberry and related plants. New York (Cornell) Agr. Expt. Sta. Bul. 395. 1917.
7. BURRILL, T. J. Blackberry and raspberry cane rust. Agr. Rev. **2** (4): 89–92. 1882.
8. COBB, N. A. Letters on the diseases of plants. Agr. Gaz. N. S. W. **14**: 627–652; 681–712; 955–986; 1057–1072. 1903.
9. DAVIS, W. H. Anthracnose of the snowberry (*Symphoricarpos albus* var. *laevigatus*). (Abst.) Phytopath. **19**: 101–102. 1929.
10. ————. Anthracnose, alternariose and Botrytis rot of the snowberry. Mycologia **23**: 159–190. 1931.
11. EDGERTON, C. W. The physiology and development of some anthracnoses. Bot. Gaz. **45**: 367–408. 1908.
12. FABRE, E. ET F. DUNAL. Observations sur les maladies régnantes de la vigne. Bul. Soc. Cent. d'Agr. Dép. de l'Hérault. **40**: 11–75. 1853.
13. JENKINS, A. E. The avocado scab organism. Phytopath. **15**: 807. 1925.
14. ————. Scab of Canavalia caused by *Elsinoë canavaliae*. Jour. Agr. Res. **42**: 1–12. 1931.

15. ————————. Lima-bean scab caused by Elsinoë. Jour. Agr. Res. **42**: 13–23. 1931.
16. ————————. Elsinoë on apple and pear. Jour. Agr. Res. **44**: 689–700. 1932.
17. ————————. Rose anthracnose caused by Sphaceloma. Jour. Agr. Res. **45**: 321–337. 1932.
18. ————————. Additional studies of species of Elsinoë and Sphaceloma. Mycologia. (In press).
19. MACOUN, W. T. Report of the horticulturist. Canada Exp. Farms Rpt. **1904**: 105–141. 1905.
20. PEGLION, V. Diagnosi di funghi parassiti nuovi. Riv. Patol. Veg. **3**: 1–14. 1894.
21. QUAINTANCE, A. L., and C. L. SHEAR. Insect and fungous enemies of the grape east of the Rocky Mountains. U. S. Dept. Agr. Farmers' Bul. 284. 1907.
22. RACIBORSKI, M. Parasitische Algen und Pilze Java's, t. 1 and 2. Herausgegeben vom Botanischen Institut zur Buitenzorg. Batavia. 1900.
23. RORER, J. B. The anthracnose of the mango. Bul. Dept. Agr. Trinidad and Tobago **14**: 164–171. 1915.
24. SACCARDO, P. A. Fungi Veneti novi vel critici vel mycologiae Venetae addendi. Ser. VII. Michelia **1**: 133–221. 1878.
25. ————————. Sylloge Fungorum, Vol. 18. 1906.
26. SCRIBNER, F. L. Notes on the orange leaf scab. Bul. Torrey Bot. Club **13**: 181–183. 1886.
27. ————————. Report on the fungus diseases of the grape vine. U. S. Dept. Agr. Bot. Div. Bul. 2. 1886.
28. ————————. Report of the mycological section. *In* Report of the Commissioner, U. S. Dept. Agr. **1886**: 95–138. 1887.
29. ————————. Report of the section of vegetable pathology. *In* Report of the Commissioner, U. S. Dept. Agr. **1887**: 323–397. 1888.
30. ————————. Fungus diseases of the grape and other plants and their treatment. 134 pp. J. T. Lovett Company, Little Silver, N. J. 1890.
31. SHEAR, C. L. The life history of *Sphaceloma ampelinum* de Bary. Phytopath. **19**: 673–679. 1928.
32. ————————, and ANNA K. WOOD. Ascogenous forms of Gloeosporium and Colletotrichum. Bot. Gaz. **43**: 259–266. 1907.
33. ————————. Studies of fungous parasites belonging to the genus Glomerella. U. S. Dept. Agr. Bur. Plant Ind. Bul. 252. 1913.
34. STEVENS, F. L., and J. G. HALL. Diseases of economic plants. Rev. ed. by F. L. Stevens. 507 pp. The Macmillan Company, New York. 1921.
35. STEVENS, H. E. Avocado diseases. Fla. Agr. Expt. Sta. Bul. 161. 1922.
36. STEWART, F. C. Notes on New York plant diseases, I. New York (Geneva) Agr. Expt. Sta. Bul. 328. 1910.
37. STONEMAN, B. A comparative study of the development of some anthracnoses. Bot. Gaz. **26**: 69–120. 1898.
38. WEINMANN, J. Maladies et ennemis de la vigne. 552 pp. J.-B. Baillière et Fils, Paris. 1907.
39. WHETZEL, H. H. The terminology of phytopathology. Proc. Intern. Cong. Plant Sci. Ithaca, 1926, **2**: 1204–1215. 1929.
40. WOLF, F. A. A little-known vetch disease. Jour. Elisha Mitchell Sci. Soc. **36**: 72–85. 1920.
41. ZELLER, S. M., and J. W. DEREMIAH. An anthracnose of Ledum caused by a species of Elsinoë. Phytopath. **21**: 965–972. 1931.

EXPERIMENTS WITH HEAD SMUT OF CORN IN WESTERN OREGON[1]

E. N. Bressman and H. P. Barss

(Accepted for publication July 20, 1932)

INTRODUCTION

Head smut of corn (*Sorosporium reilianum* (Kühn) McAlp.) has been observed in western Oregon for many years. It is of interest to note, however, that the disease, although widely scattered, does not occur in all places where corn is grown in this region but is localized in certain areas where the smut may be very severe in particular fields, while it is absent or unnoticed in the intervening areas even though soil and climate are very much alike and similar varieties of corn are grown.

In 1930 a serious outbreak ruined a field near Salem, attacking 90 per cent of the plants. This directed our attention to the possible serious economic consequences of the disease and led to the trials reported herein.

PREVIOUS RESEARCH

This head smut was first found in 1868 in Egypt and has since been noted in many parts of the world. It was first reported on the Pacific Coast from the State of Washington, in 1920, by Dana and Zundel[2] and from California in the same year by Mackie.[3] Among the most extensive studies of the disease are those reported by A. A. Potter[4] in 1914 and by Reed, Swabey, and Kolk[5] in 1917. The more important literature on the disease has been discussed in their contributions and will not be reviewed here.

The work of various investigators, particularly that of Reed and his associates, indicates that the head smut of corn does not readily attack sorghums, and *vice versa*, although the strains are morphologically indistinguishable.

Several workers have demonstrated that the disease may be transmitted to a certain extent by seed-borne spores, but Potter[4] and others have shown

[1] Published as Technical Paper No. 187 with approval of the Director, Oregon Agricultural Experiment, Station. Contribution from the Departments of Farm Crops and Botany.

[2] Dana, B. F., and G. L. Zundel. Head smut of corn and sorghum. Washington Agr. Expt. Sta., Pop. Bul. 119. 1920.

[3] Mackie, W. W. Head smut in sorghum and maize. Phytopath. 10: 307–308. 1920.

[4] Potter, A. A. Head smut of sorghum and maize. Jour. Agr. Res. 2: 339–372. 1914.

[5] Reed, G. M., Marjorie Swabey, and Laura Alma Kolk. Experimental studies on head smut of corn and sorghum. Bul. Torrey Bot. Club 54: 295–310. 1927.

that the chief source of infection lies in smut-contaminated soil. Seedlings are attacked in the early stages of their development and the parasite becomes systemic, growing up within the plant and generally transforming some or all of the flowering parts into smut galls. Numerous instances are on record, however, where, instead of producing smut masses in the tassels and ears, the presence of the parasite induces the formation of abnormal vegetative proliferations in place of normal floral organs. It has also been noted by McAlpine[6] and by Reed, Swabey, and Kolk[7] that the tassels may sometimes show no smut on corn plants having infected ears, whereas if smut appears in the tassel, all the ears are generally smutted. These phenomena were observed in the trials reported herein.

Thus far, no satisfactory control measures for head smut seem to have been developed. Seed treatment of smut-contaminated grain has been reported effective in eliminating this source of infection, but no practical method has yet been worked out for the elimination of soil-borne infection, a vastly more important source of the disease. Potter,[8] however, suggests what he characterizes as "a possible, though very doubtful and as yet untried specific measure for prevention, *i.e.*, a treatment of the soil about the seed at planting time in some such way as is done for onion smut."

PRESENT INVESTIGATIONS

In view of the seriousness of the head smut of corn on various Oregon farms, it was thought desirable to carry out certain infection studies by means of greenhouse and field trials, using methods similar to those of previous workers, and, in addition, to try out various materials for seed treatment as well as for treatment of the soil at planting time, as suggested by Potter. The results here presented confirm and extend the knowledge contributed by others, but they are based on experiments so limited in scope that they cannot be considered conclusive in all particulars.

GREENHOUSE TRIALS IN 1931

Seed Treatment Experiments With Corn. Seed of the variety Minnesota 13, from the 1930 crop on the Oregon State College Farm, where head smut is unknown, was thoroughly coated with head-smut spores obtained from the badly infested field near Salem, referred to in the introduction. The contaminated seed was then divided into 8 lots, 6 of which were treated with various dust materials by shaking the seed vigorously with a liberal excess of the dust. With another lot, formaldehyde was used at the strength of 1 oz. to 1 gal. in a 15-minute soak. The treated lots and the nonsmutted and smutted controls were then planted in noncontaminated

[6] McAlpine, D. The smut of maize and its treatment. Jour. Dept. Agr. Victoria 8: 290–298. 1910.

[7] See footnote 5.

[8] See footnote 4.

soil in a deep greenhouse bench on January 5. One lot of clean seed was similarly planted, after which the surface of the soil was given a liberal coating of smut spores. The soil surface was kept moist by daily watering. Greenhouse temperatures ranging from 70 to 90° F. during the day prevailed throughout germination and growth. By extending the daily exposure to light through the use of electric illumination, these plants were forced to produce tassels and ears by May 10. The results of these trials are reported in table 1.

TABLE 1.—*Results of various treatments of seed of Minnesota No. 13 corn, artificially contaminated with spores of Sorosporium reilianum. Greenhouse trials, Corvallis, 1931*

Treatment	Normal stalks	Stalks with tassel and ears smutted	Stalks with only ears smutted	Stalks with abnormal ears only	Total stalks
Clean seed—check	8	0	0	0	8
Smutted seed—check	2	2	14	12	30
Copper carbonate (50%)	4	0	0	0	4
Formaldehyde (1 oz. to 1 gal.)	3	0	1	0	4
Improved Semesan Jr.	0	0	3	1	4
Landplaster (powdered gypsum)	4	0	0	0	4
Corona Merko (mercuric)	0	0	2	2	4
Field's Corn Dust (copper)	1	0	1	2	4
Paraformaldehyde (powdered)	4	0	0	0	4
Clean seed, smut on top soil	4	0	0	0	4
Totals	30	2	21	17	70

These tests clearly demonstrate that corn may become infected from seed-borne spores, since artificial smutting of the seed resulted in evident infection in 28 out of 30 plants. Clean seed produced only clean plants, even where a layer of smut was placed on the soil surface. All of the seed treatments appeared to reduce or eliminate the infection, with the exception of Improved Semesan Jr. and Corona Merko, neither of which, of course, has been recommended for smut prevention, although commonly used on corn. These tests are too limited to warrant any decision as to the relative merits of the different materials employed.

It is interesting that out of 40 infected corn plants only 2 showed smutted tassels, while 17 disclosed the presence of the parasite only by the production of abnormal vegetative proliferation in the ears without the formation of smut masses. (Fig. 1.)

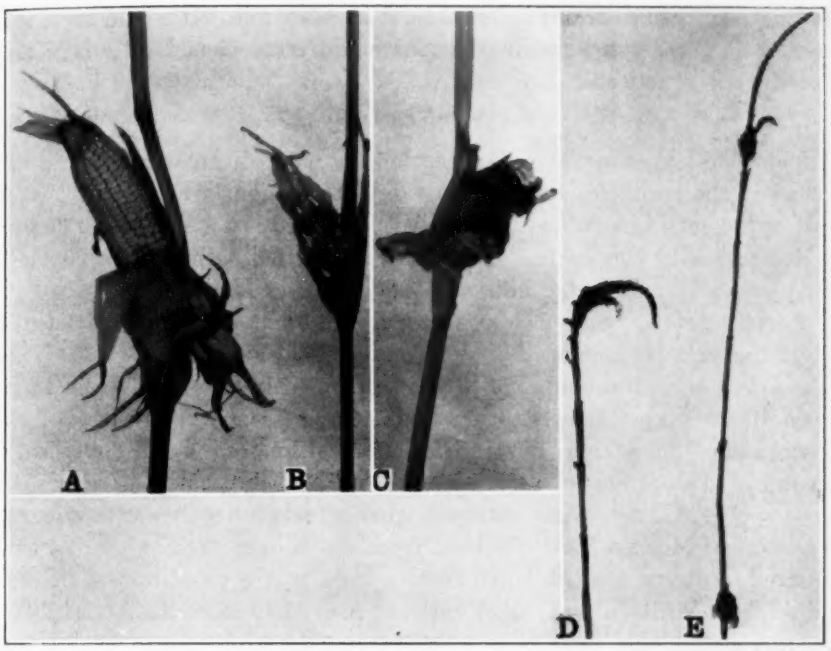

FIG. 1. Effect of *Sorosporium reilianum* on corn in greenhouse trials. A. Normal ear. B. Abnormal ear. C. Smutted ear. D. Stalk with smutted tassel. E. Stalk with smutted tassel and ear. In D and E leaves have been removed.

No conjecture is offered as to why the plants came through healthy in the lot where smut was placed on the soil surface at planting time, especially since considerable care was taken to keep the soil surface in a moist condition and the atmosphere of the greenhouse humid.

Infection Trials With Head Smut of Corn on Sorghum. Seed of 6 varieties of sorghum was obtained from G. M. Reed, of the Brooklyn Botanic Garden, and coated with head-smut spores from the same source as the material used for the above experiment with corn. The contaminated seed was planted in the greenhouse on February 17. The sorghum headed by July 28.

Dr. Reed reports on the relative susceptibility of these varieties to the sorghum strain of *Sorosporium reilianum* as follows: Black Amber sorgo (No. 48) susceptible; brown durra (No. 74) susceptible; Red Amber sorgo (No. 232) susceptible; Dawn Kafir (No. 186) resistant; Dwarf Yellow milo (No. 124) resistant; Manchu kaoliang (No. 191) resistant.

Although from 19 to 24 plants of each variety, 128 in all, matured, not a single plant showed any sign of head smut. This result confirms the prevalent opinion that the sorghums are not readily attacked by the ordi-

nary corn-infecting strains of *Sorosporium reilianum*. It would seem safe, therefore, to use sorghum in rotations on infested corn land where such procedure is practicable.

FIELD TRIALS IN 1931

The field experiments on susceptibility and soil treatment were conducted in the cornfields near Salem, Oregon, previously referred to, where most of the corn was ruined by the head smut in 1930. The soil is mapped as Holcomb silty clay loam. The corn was planted on May 8 in 26 single-row plots, 5 kernels to the hill.

Variety Trials. Six varieties of corn were planted without treatment to determine relative susceptibility in naturally contaminated soil. These were as follows: Minnesota No. 13 grown for nearly 25 years at the Oregon Agricultural Experiment Station and coming from a crop free of this disease; the "Schucking" strain, similar to Minnesota No. 13, obtained in the fall of 1930 from an infested field and probably carrying some seed-borne spores; Golden Glow obtained from a farm where no smut occurred in the crop; McKay Dent obtained from the college farm where no smut occurred; Putnam Red obtained from a farm where no smut was present; and African White, a flinty dent variety received from South Africa direct. Whether or not this came from a smut-free field is unknown. The results are shown in table 2.

TABLE 2.—*The results of attack by Sorosporium reilianum on 6 varieties of corn planted without treatment in smut-contaminated soil at Salem, Oregon, in 1931*

Variety	Stand	Total stalks	Disease-free stalks	Stalks with tassels and ears smutted	Stalks with only ears smutted	Stalks with abnormal ears only	Diseased stalks
	Per ct.						*Per ct.*
Minnesota No. 13	99	95	76	13	4	2	20
"Schucking"	95	92	57	20	13	2	38
Golden Glow	96	93	61	15	15	2	34
McKay Dent	83	80	65	4	8	3	19
Putnam Red	82	79	58	13	5	3	27
African White	78	75	50	17	2	6	33
Totals	—	514	367	82	47	18	28.6

The results shown in table 2 do not disclose any variety with marked resistance to head smut. The limited nature of the test and lack of replica-

tion do not allow any decision as to whether the different percentages of smut attack on the different varieties are true indications of relative susceptibility.

Field Treatment Trials. Two of the varieties, Minnesota No. 13 and the "Schucking" strain were given 10 treatments at the time of planting. Copper carbonate, containing 50 per cent copper; Corona Merko, a mercury-containing powder; Field's Corn Dust, a copper-containing powder; and Improved Semesan Jr., an organic-mercury dust, were applied in excess as dusts on the seed before planting. All of these dusts stick to the seed readily. Smuttox, composed of diatomaceous earth carrying 4 per cent formaldehyde; agricultural ground sulphur; and Superpoppy sulphur, which contains a considerable proportion of black gas by-product sulphur; were applied by placing about a level teaspoonful on the kernels after they were put in the hills. The hills were then covered. Formaldehyde was used at the strength of 1 oz. of the commercial (about 40 per cent) solution to 1 gal. of water. Germ-O-No liquid was diluted 1 to 10, and Garden Volck, a refined oil emulsion, was diluted 1 to 8. In these 3 liquid treatments the corn was planted and covered with the soil, after which the liquid was carefully poured on top of the soil. About 1 teacupful was applied to each hill, which was sufficient to wet the ground down to the kernels.

About 2 weeks after planting, stand counts were made and the hills that had more than 3 stalks were reduced to a uniform stand of 3. The growth for the first month was rather slow because of dry conditions. Timely rains in mid-June, however, were followed by rapid growth. The plants were left undisturbed until fall when counts of smutted, abnormal and normal tassels and ears were obtained. The results are reported in table 3.

The results presented in table 3 show that none of the treatments as carried out gave clear evidence of practical effectiveness for head-smut prevention in contaminated soil. Even the formaldehyde solution, so effective when applied to soil for onion-smut control, produced no definite evidence of protection as compared with the control rows. In the test with the "Schucking" strain, copper carbonate produced marked injury to stand, and additional trials have shown that, in excess quantity, copper carbonate inhibits the growth of the seminal roots. The formaldehyde-carrying dust, Smuttox, produced severe reduction in stand in both varieties. At the same time it afforded no protection. In passing it may be noted that the plots treated with Corona Merko, although showing considerable smut, appeared greener and more vigorous than any of the others from the seedling stage to harvest.

Taking the totals from all the field trials, which included 2,034 corn plants, 674, or 33 per cent, were diseased. Of these, 49 per cent showed both tassels and ears smutted, 40 per cent showed only ears smutted, while

TABLE 3.—*Field trials of the results of seed and soil treatments with corn against Sorosporium reilianum, conducted at Salem, Oregon, in 1931*

Treatment	Stand 2 weeks after planting	Total stalks matured	Disease-free stalks	Stalks with tassels and ears smutted	Stalks with smutted ears only	Stalks with abnormal ears only	Diseased stalks
			Minnesota No. 13				
	Per ct.						*Per ct.*
Copper carbonate	100	97	79	10	5	3	19
Corona Merko	89	86	53	16	14	3	37
Field's Corn Dust	77	74	48	13	12	1	35
Improved Semesan Jr.	100	99	61	18	15	5	38
Smuttox	30	29	20	4	4	1	31
Agricultural ground sulphur	93	90	66	14	6	4	27
Superpoppy sulphur	91	88	53	17	16	2	39
Formaldehyde	85	82	63	7	10	2	23
Germ-O-No	64	62	42	13	3	4	32
Garden Volck	83	80	65	10	4	1	19
Nontreated check	99	95	76	13	4	2	20
Totals	—	882	626	135	93	28	29
			"Schucking" Strain				
Copper carbonate	52	50	44	3	2	1	12
Corona Merko	100	99	72	15	9	3	27
Field's Corn Dust	72	70	56	5	8	1	20
Improved Semesan Jr.	92	89	53	16	17	3	40
Smuttox	34	33	18	8	5	2	45
Agricultural ground sulphur	72	70	35	10	23	2	50
Superpoppy sulphur	100	100	56	26	13	5	44
Formaldehyde	59	57	38	7	9	3	33
Germ-O-No	78	75	32	12	27	4	57
Garden Volck	93	90	39	22	25	4	57
Nontreated check	95	92	57	20	13	2	38
Totals	—	825	500	144	151	30	39

11 per cent showed abnormal vegetatively proliferating ears only. In some instances partly smutted ears and tassels were observed. Wherever smut showed in a tassel, however, all ears on the plant showed smut. The proportionate appearance of diseased tassels and ears and of abnormal ears in the field experiments is distinctly in contrast to the results met with in the greenhouse trials where out of 40 diseased plants only 5 per cent showed smutted ears only, and 43 per cent showed abnormal ears only.

There is evidence to indicate that head smut has been present on corn in Oregon for a good many years. At a meeting of corn growers held in 1931 at the experimental field, a neighboring farmer stated that he had

seen smut in this field as long ago as 35 years. Other fields in this vicinity also showed head smut in 1931. One would expect the disease to be prevalent throughout the Willamette Valley; yet, at a meeting of corn growers held at Oregon City some 40 miles to the north of this experimental field, many in attendance had grown corn for 20 years or more, but none had observed this disease.

SUMMARY

Sorosporium reilianum, head smut of corn, is found destructive in certain localities of western Oregon, while it is absent from others. Experiments were conducted with this disease in the greenhouse and in the field in 1931. The results demonstrated that the disease may be transmitted to a certain extent by seed-borne spores; that the local strain of head smut of corn was not successfully transmitted to 6 varieties of sorghum, whereas it produced abundant infection on 6 varieties of corn; that various seed treatments can be used to prevent seed-borne infection, but that no seed or soil treatment included in these tests was effective in preventing infection in smut-contaminated soil.

OREGON AGRICULTURAL EXPERIMENT STATION,
CORVALLIS, OREGON.

HOW PLANT PATHOLOGY CAN CONTRIBUTE TO A LAND-UTILIZATION PROGRAM

NEIL E. STEVENS

(Accepted for publication July 19, 1932)

The suggestion that an increase in our knowledge of the distribution of plant diseases and their relative severity in different localities is of more immediate economic significance than a further increase in our knowledge of the methods of disease control seems like an indictment of all pathological teaching and present practices. Yet, this is the logic of events, and a plant pathology that neglects economic conditions can lay little claim to being practical. To appreciate how great a change has occurred in our economic thinking it is necessary merely to refer to serious statements of 8 or 9 years ago to the effect that, about 1906, the United States entered a period when population increased faster than agricultural production, that our exportable excess was decreasing, and that within 15 or 20 years our consumption would equal our production. These statements sound like counsels from another civilization.

THE PRESENT PROBLEM

The outstanding features of the present agricultural situation have been widely discussed and there is general agreement as to the need for a planned use of our land resources. The major feature of this program in relation to agricultural land was clearly stated last October by Franklin D. Roosevelt, then Governor of New York, in an article that includes the sentence—"Today in spite of the great increase in our population, the farm acreage problem in the United States is more a problem of reducing our acreage on a sound scientific basis—reducing it where it ought to be reduced—than of expanding it."[1]

PLANT PATHOLOGY AND ACREAGE ADJUSTMENT

That plant-disease information would be important in an adequately planned agricultural adjustment is obvious to the well informed. It is almost equally obvious that for the greatest effectiveness there will be necessary some reorientation of the studies of professional plant pathologists. It may be taken for granted that in any acreage adjustment the "high-cost" acres should be first abandoned. It is evident that a given area may properly belong in the high-cost group because of disease, as well as soil, factors. Any region in which the diseases of a particular crop are severe

[1] Roosevelt, F. D. Planning use of land for farm, forest and highway. Louisiana Conservation Rev. 2 (11): 22–23. Oct., 1932.

or particularly hard to control becomes necessarily a high-cost area for that crop.

In his report to the President for 1931 (pages 37–38) the Secretary of Agriculture urges an economic classification of our land resources and widespread dissemination of information concerning the uses for which particular classes of land are economically adapted on the ground that such a classification would make it easier to discourage ill-advised expansion and lessen the risks of new settlement. A classification of crop areas with reference to severity of plant diseases and the difficulty of their control would have similar usefulness.

The compilation by plant pathologists of a map of the United States showing areas in which disease control on certain crops has proved particularly difficult should be well within our scientific possibilities. Whether it will prove to be a practical possibility may be a quite different matter. It admittedly runs counter to most of our previous practice, as well as to our American psychology. A disease particularly difficult to control offers to the pathologist, of course, a particularly attractive problem. Hitherto, an attempt to grow a crop in a region for which it was not well suited has been accepted by plant pathologists as a challenge to their best efforts.

To suggest the abandonment of a crop project sounds like a council of defeat and is correspondingly repugnant to our temperaments. It is, however, no different essentially from a soil survey that names given areas as being unsuited to profitable cultivation. The suggestion of a nation-wide mapping of areas that, under present conditions, are not worth cultivating sounds like a defeatist policy. It is, however, quite the contrary. The day of expansion of crop production in the United States has definitely passed, perhaps, for a long time. We may well be entering a period of contraction of acreage. Under present economic conditions some lands may even be forced out of production. Obviously, a planned acreage adjustment is preferable to one forced by bankruptcy. And one in which the knowledge of the plant pathologists shall be utilized is better than one planned without reference to plant diseases or to their control.

A few examples of the way in which adequate plant-disease information might have served to "discourage ill-advised and unnecessary expansion" may be cited in the field of small fruits. Information as to the probable difficulty of disease control was already in the hands of plant pathologists, which, if it could have been followed by growers, would have prevented costly and disastrous attempts to grow Vinifera grapes in Texas, Euvitis grapes in Florida and the coastal regions of the Carolinas, raspberries in eastern South Carolina, currants in eastern Maryland, and cranberries in extreme southern New Jersey.

Fortunately, for immediate progress, it is not necessary to plan a crop-loss map to be at once published and widely disseminated. The first steps will be the assembling of available information and the developing of methods of securing better information of this type. It has been frequently pointed out that present economic difficulties offer a challenge to our civilization equal to that of the World War. The present need for classified information regarding plant diseases and their control would appear to offer an opportunity for American plant pathologists second only to that so effectively met by them during the period of the World War.

BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

PHYTOPATHOLOGICAL NOTES

A Hitherto Unreported Blight of the Juglandaceae.—At Stamford, Connecticut, I have a collection of trees. Some of them are employed for hybridizing purposes. In this collection there are various hybrids that have been obtained during the past 35 years.

This year I planned to obtain hybrids between the English walnut, *Juglans regia,* and the butternut, *J. cinerea.* The English walnut was of the Webb variety, which has enormous nuts, and the butternut was one of many seedling natives. In the last week in May I bagged pistillate flowers of the English walnut and then waited for the aments to ripen their pollen and fall from the tree. This would naturally occur at about the time when butternut pollen would be viable. On the morning of the day when the English walnut pollen should have ripened the anthers had all blackened and the pollen was destroyed. Specimens of the ruined aments were sent to the Connecticut Agricultural Experiment Station at New Haven, Connecticut. G. P. Clinton, the Plant Pathologist, expressed the opinion that the blight was bacterial in its nature. A few days later I picked an ament from the butternut tree that was to furnish pollen for the English walnut. In my opinion the butternut would shed pollen at daylight on the following day. The plucked ament was tossed upon the ground. At about 8 o'clock on the following day all of the anthers on the butternut tree were ruined by the blight and were black. The ament that had been tossed upon the ground was shedding its pollen and was not blighted.

During the next few days blight had attacked the anthers of all butternuts, their hybrids, and their parthenogens. It had attacked the anthers of 5 cultivated varieties of the black walnut, *Juglans nigra,* and of several varieties of the Japanese walnut, *J. sieboldii.* No male flowers remained upon *J. sinensis* or upon *J. sibirica.* Unfortunately, I did not examine flowers of a number of species and varieties of the hickories because they did not enter into my hybridization plans for this year; but *Hicoria pecan* aments of 3 varieties were found blighted, and it was observed that *H. ovalis* was the last one of the hickory group to flower and to blight.

Incidentally, some of the butternut aments, which were mostly blighted but bore a little pollen, were carried to the English walnut tree and shaken over pistillate flowers. The appendages of these flowers turned partly or wholly brown the next day. They had not been attacked by blight originally, but the brownish effect seemed to have resulted from contact with blighted aments.

At this date of writing, August 26, many of the walnut trees of several species and varieties that should be heavily laden with nuts have none at

all. Others have a few clusters, as though some pollen had acted very locally on the tree.

The blight seemed to have one notable peculiarity. It apparently did not attack pollen-bearing anthers except on the night when they were ready to shed pollen the following day, and it then destroyed all or nearly all on any given tree on that particular night. Sometimes, the process seemed to be extended over 2 or 3 nights, so that a little pollen escaped from a few flowers or from parts of a few flowers during 1 or 2 days.

During about 35 years of hybridizing experiments I had never previously noticed this blight. If it had been present it was not in sufficient degree to attract attention.—ROBERT T. MORRIS, M.D., Stamford, Connecticut.

A Disorder of Persian (English) Walnuts Grafted on Black-walnut Stocks, Resulting in Girdling.—A disorder of Persian (English) walnuts grafted upon black-walnut rootstock is causing considerable concern to Oregon walnut growers in the Willamette Valley. The trouble was first noted in 1924 in a 15-year-old grafted Franquette orchard near Dundee, Oregon. Since then it has either been found or reported from several other orchards of the Franquette variety in western Oregon.

The first evidence of this trouble is shown by poor shoot growth and by premature shedding of the leaves on a part or all of the tree. As a rule, suckers from the black-walnut rootstock appear at the same time. Death usually follows 3 or more years after the first symptoms are noted in the aerial portions of the tree. The devitalization and death of the tree are apparently caused by girdling brought about by the failure of the newly formed xylem tissues of the Persian top to unite with the black-walnut rootstock. As the new cells of the xylem are laid down by the cambium the two kinds of tissue no longer unite. A dark discolored layer of a corky nature is formed between the two kinds of wood. At first it may be only a fraction of a millimeter in thickness but, in the later stages, it may be as much as $\frac{1}{4}$ in. thick. Only a few inches of the new tissue at the point of union may be affected the first year, but, eventually, the whole circumference of the tree is affected in a like manner. In most cases death of the tree ensues 3 years after the first symptoms are evident. The girdling of the tree is apparently preceded by the presence of water-soaked, chocolate-color spots in the bark of the rootstock at or below the point of union. In the early stages of the trouble these spots are very small and usually isolated. Later, they enlarge to several inches in diameter and often several of these spots merge, forming a large area of dead tissue. In some instances the first visible symptom of the disorder seems to consist of a discolored narrow strip of tissue in the vicinity of the union encircling the tree, but not penetrating to any depth into the bark. In such instances no evidence of a lack of union has been

found by cutting through the bark. In cases where connecting tissue extended from this outer strip into the woody tissue a break in the union always occurred. The trouble has so far been found only on the Franquette variety grafted on a black-walnut rootstock, of which the individual rootstocks are apparently *Juglans hindsi* (Sarg.) (Hinds or Northern California black walnut) or hybrids of other species with this one.

The cause of this disorder is not definitely known. A number of attempts have been made by the junior writer to isolate some parasitic organism from the discolored tissues but to date no infectious microorganism has been found to be constantly associated with the trouble. It appears, therefore, that this disorder is of nonparasitic nature.—C. E. SCHUSTER, Bureau of Plant Industry, United States Department of Agriculture, Corvallis, Oregon; and P. W. MILLER, Division of Horticultural Crops and Diseases, United States Department of Agriculture, Corvallis, Oregon.

THE CANADIAN PHYTOPATHOLOGICAL SOCIETY

Advance Notice of Meeting

Arrangements are being completed for the holding of the 1933 meeting of the Society at Regina, Sask., in July, in conjunction with the World's Grain Fair and the C. S. T. A. Convention. A committee under the chairmanship of Dr. P. M. Simmonds, Saskatoon, has been appointed to look after local arrangements.